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# Effects of a Eucaloric Low Glycemic Index Diet on Insulin Sensitivity and Intramyocellular Lipid Content in Adults with Abdominal Obesity

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**Effects of a Eucaloric Low Glycemic Index Diet on Insulin Sensitivity and Intramyocellular  
Lipid Content in Adults with Abdominal Obesity**

by

Angela Marie Kochan

A thesis submitted in conformity with the requirements  
for the degree of Doctor of Philosophy

Graduate Department of Nutritional Sciences  
University of Toronto

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Doctor of Philosophy

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University of Toronto

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## **Abstract**

Individuals with abdominal obesity are at higher risk for developing type 2 diabetes, predisposing cardiovascular events and insulin resistance. Low glycemic index (GI) diets may be beneficial in the management of insulin resistance. Insulin resistance is associated with increased intramyocellular lipid (IMCL) content as measured by proton nuclear magnetic resonance spectroscopy ( $^1\text{H}$ -MRS). The primary objective of this thesis was to determine whether a low GI diet can improve insulin sensitivity by reducing IMCL of skeletal muscle. One hundred and twenty-one male and female participants aged 30 to 70 years (mean $\pm$ SD, 53 $\pm$ 10) with abdominal obesity, entered a 4 to 6 week weight-maintaining, low-fat dietary advice run-in phase. Of the 121 eligible participants, 95 completed the run-in phase and were randomly assigned to either a low-GI (LGID, n=48) or high-GI diet (HGID, n=47) for 24 weeks. Participants underwent a 75g oral glucose tolerance test (OGTT) and had soleus-muscle IMCL measured by  $^1\text{H}$ -MRS at the beginning and end of the intervention period. Insulin sensitivity was assessed by the homeostatic model assessment index (HOMA) and the insulinogenic index (ISI) was calculated for insulin secretion. At the end of the run-in phase, there were significant reductions in serum total-, LDL-, and HDL-cholesterol (all,  $p<0.0001$ ) and an increase in fasting

plasma glucose ( $p<0.05$ ). In 57 participants who wore a continuous glucose monitoring system for 24 hours during the run-in period, a total of 30% ( $p<0.001$ ) of the variation in the incremental area under the blood glucose curve after self-selected breakfast meals was explained by GI. After 24 weeks, diet GI was significantly lower in the LGID than HGID group ( $55.5\pm3.1$  vs  $63.9\pm3.1$ ,  $p<0.0001$ ). Plasma glucose 60 minutes after the OGTT was significantly lower on the LGID than at baseline ( $p<0.05$ ) and there was a non-significant trend towards an increase in ISI ( $p=0.07$ ). On the HGID, ISI increased significantly from baseline ( $p<0.01$ ). It is concluded that the LGID reduced 60 minute plasma glucose but did not significantly affect IMCL or insulin sensitivity in individuals with abdominal obesity.

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## **PUBLICATIONS ARISING FROM DISSERTATION**

### **Publications:**

Kochan AM, Wolever TMS, Chetty VT, Anand SS, Gerstein HC, Sharma AM. Glycemic index predicts individual glucose responses after self-selected breakfasts in free-living, abdominally obese adults. *Journal of Nutrition* 2012;142:27-32.



## ABBREVIATIONS

BMI	Body Mass Index
BP	Blood Pressure
cm	centimetres
CT	Computed Tomography
CVD	Cardiovascular Disease
EMCL	Extramyocellular Lipids
FFA	Free Fatty Acids
GI	Glycemic Index
GL	Glycemic Load
HDL-C	High Density Lipoprotein Cholesterol
HGID	High Glycemic Index Diet
$^1\text{H-MRS}$	Proton Nuclear Magnetic Resonance Spectroscopy
HOMA	Homeostatic Model Assessment Index
HR	Heart Rate
iAUC	Incremental Area Under the Glucose Response Curve from the Oral Glucose Tolerance Test
$\text{iAUC}_{\text{breakfast}}$	Incremental Area Under the Glycemic Response Curve for 2 hours after Breakfast
$\text{iAUC}_{\text{OGTT}}$	Incremental Area Under the Glycemic Response Curve for 2 hours after 75 grams Oral Glucose
IDF	International Diabetes Federation
igAUC	Incremental Area Under the Curve for Plasma Glucose from the Oral Glucose Tolerance Test
iiAUC	Incremental Area Under the Curve for Plasma Insulin from the Oral Glucose Tolerance Test

IGT	Impaired Glucose Tolerance
IFG	Impaired Fasting Glucose
IMCL	Intramyocellular Lipids
ISI	Insulinogenic Index
kcal	kilocalories
LDL-C	Low Density Lipoprotein Cholesterol
LGID	Low Glycemic Index Diet
mmol/L	millimoles per litre
NCEP, ATP III	National Cholesterol Education Program, Adult Treatment Panel III
NEFA	Non-esterified Fatty Acids
OGTT	Oral Glucose Tolerance Test
pmol/L	picomoles per litre
ppm	parts per million
SAT	Subcutaneous Adipose Tissue
μU/ml	microunits per millilitre
VAT	Visceral Adipose Tissue
VLDL	Very Low Density Lipoprotein
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist to Hip Ratio

**CHAPTER 1**  
**INTRODUCTION**

## **1. Introduction**

Abdominal obesity, characterized by the accumulation of visceral adipose tissue, is now recognized as an independent risk factor for the development of cardiovascular disease (CVD), type 2 diabetes, metabolic syndrome and insulin resistance (1, 2, 3, 4, 5). Insulin resistance, defined as an impairment of insulin action on glucose metabolism is a complex, multi-organ dysfunction that is brought about by a multitude of genetic and environmental factors, such as a sedentary lifestyle and high fat diets leading to excess energy intake (6). Although not fully understood, it has been suggested that individuals with abdominal obesity cannot store lipids effectively in subcutaneous fat, which in turn leads to an increased delivery of fats to organs other than adipose tissue such as the liver, pancreas and skeletal muscle, causing insulin resistance in these tissues (7, 8).

Skeletal muscle facilitates insulin-stimulated glucose uptake and is the main contributor to glucose homeostasis in the body (6, 9). Research has shown that the accumulation of triglyceride in muscle is correlated with insulin resistance independently of visceral fat (10). Muscle triglyceride content is categorized as extramyocellular lipids (EMCL, lipids stored between muscle fibres) and intramyocellular lipids (IMCL, lipids stored within the muscle cell) (11). Muscle triglyceride content can be assessed by examination from proton nuclear magnetic resonance spectroscopy ( $^1\text{H}$ -MRS), a non-invasive quantification of muscle fat content in human muscle that can identify the relative contributions of IMCL (11). Research has shown a positive relationship between the accumulation of IMCL content and insulin resistance when measured by  $^1\text{H}$ -MRS demonstrating that IMCL is an indicator of whole body insulin sensitivity (12, 13, 14, 15).

Evidence supports the role of carbohydrates in influencing insulin sensitivity by improving insulin-stimulated glucose uptake by adipocytes when following a low glycemic index diet (16, 17, 18). The glycemic index (GI) was developed as a classification of the blood glucose-raising potential of carbohydrate containing foods (19). The GI is defined as the incremental area under the blood glucose response curve (iAUC) after consumption of a 50g available-CHO portion of a test food expressed as a percentage of that after ingestion of 50g oral glucose (20). Low GI carbohydrates are slowly digested and release glucose gradually into the blood stream and therefore may suppress hepatic glucose production and non-esterified fatty acid (NEFA) release from adipocytes, creating a more insulin-sensitive condition (21, 22). This in turn, may reduce IMCL storage by promoting skeletal muscle glucose uptake (23). It still remains unclear whether a low GI diet can reduce IMCL stores in the muscles of individuals with abdominal obesity, a population at risk for developing insulin resistance and type 2 diabetes.

The primary objective of the research outlined in this dissertation was to determine whether a low GI diet would reduce IMCL stores thereby improving insulin sensitivity in individuals with abdominal obesity. A secondary objective of this research was to determine whether a low GI diet would improve insulin sensitivity and glucose tolerance in individuals with abdominal obesity.

## 1.1 References

1. Miyazaki Y, Glass L, Triplitt C, Wajcberg E, Mandarino LJ, DeFronzo RA. Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus. *American Journal of Physiology, Endocrinology and Metabolism* 2002;283:E1135-E1143.
2. Ross R, Aru J, Freeman J, Hudson R, Janssen I. Abdominal adiposity and insulin resistance in obese men. *American Journal of Physiology, Endocrinology and Metabolism* 2002;282:E657-E663.
3. Ross R, Freeman J, Hudson R, Janssen I. Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. *Journal of Clinical Endocrinology and Metabolism* 2002;87:5044-5051.
4. Carr DB, Utzschneider KM, Hull RI, Kodama K, Retzlaff BM, Brunzell JD, Shofer JB, Fish BE, Knopp RH, Kahn SE. Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes* 2004;53:2087-2094.
5. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith Jr SC, Spertus JA, Costa F; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735-2752.
6. Gastaldelli A, Natali A, Vettor R, Corradini SG. Insulin resistance, adipose depots and gut: Interactions and pathological implications. *Digestive and Liver Disease* 2010;42:310-319.
7. Shulman GI. Unraveling the cellular mechanism of insulin resistance in humans: New insights from magnetic resonance spectroscopy. *Physiology* 2004;19:183-190.

8. McGarry JD, Dobbins RL. Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 1999;42:128-138.
9. Stefanyk LE, Dyck DJ. The interaction between adipokines, diet and exercise on muscle insulin sensitivity. *Current Opinion in Clinical Nutrition and Metabolic Care* 2010;13:255-259.
10. Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes* 1997;46:1579-1585.
11. Machann J, Haring H, Schick F, Stumvoll M. Intramyocellular lipids and insulin resistance. *Diabetes, Obesity and Metabolism* 2004;6:239-248.
12. Krssak M, Petersen KF, Dresnar A, DiPietro L, Vogel SM, Rothman DL, Shulman GL, Roden M. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a  $^1\text{H}$  NMR spectroscopy study. *Diabetologia* 1999;42:113-116.
13. Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, Haring HU. Association of increased intramyocellular lipid content with insulin resistance in lean non-diabetic offspring of type 2 diabetic subjects. *Diabetes* 1999;48:1113-1119.
14. Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT. Measurement of intracellular triglyceride stores by  $^1\text{H}$  spectroscopy: validation in vivo. *American Journal of Physiology, Endocrinology and Metabolism* 1999;276:E977-E989.
15. Kelly DE, Goodpaster BH. Skeletal muscle triglyceride. An aspect of regional adiposity and insulin resistance. *Diabetes Care* 2001;24:933-941.

16. Frost G, Keogh B, Smith D, Akinsanya K, Leeds A. The effect of low-glycemic carbohydrate on insulin and glucose response in vivo and in vitro in patients with coronary heart disease. *Metabolism* 1996;45:669-672.
17. Frost G, Leeds A, Trew G, Margara R, Dornhorst A. Insulin sensitivity in women at risk of coronary heart disease and the effect of a low glycemic diet. *Metabolism* 1998;47:1245-1251.
18. Rizkalla SW, Taghrid L, Laromiguire M, Huet D, Boillot J, Rigoir A, Elgrably F, Slama G. Improved plasma glucose control, whole-body glucose utilization, and lipid profile on a low-glycemic index diet in type 2 diabetic men. *Diabetes Care* 2004;27:1866-1872.
19. Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 1981;34:362-366.
20. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. *American Journal of Clinical Nutrition* 1991;54:846-854.
21. Wolever TMS, Bentum-Williams A, Jenkins DJA. Physiological modulation of plasma free fatty acid concentrations by diet: Metabolic implications in nondiabetic subjects. *Diabetes Care* 1995;18:962-970.
22. Thorburn A, Muir J, Proietto J. Carbohydrate fermentation decreases hepatic glucose output in healthy subject. *Metabolism* 1993;42:780-785.
23. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes and Metabolism Reviews* 1998;14:263-283.



**CHAPTER 2**  
**LITERATURE REVIEW**

## **2. Literature Review**

### **2.1 Obesity**

#### **2.1.1 Obesity Definition**

Obesity is a complex disease characterized by an excessive accumulation of fat in adipose tissue (1). It is estimated that 23.1% (CI 21.7 to 24.6) of adult Canadians are obese (2), with consistent trends in different parts of the world (3). Obesity is the result of a positive energy balance where food intake is greater than the body's ability to utilize the food as energy (1). The excess energy, in the form of triglycerides, is stored in adipocytes which increase in size and weight (hypertrophic obesity) and number (hyperplastic obesity) (4). The excessive accumulation of adipose tissue leads to an undesirable weight gain to the extent that health may be adversely affected (1). Health risk factors and comorbidities associated with obesity include cardiovascular disease (CVD) (5, 9), stroke (6, 7, 9), hypertension (5, 8), type 2 diabetes (10, 11, 12, 13), sleep disordered breathing (14), kidney disease (15) certain cancers such as colon (16), ovarian (17), breast (18), and endometrial cancers (19), gallbladder disease (20), and musculoskeletal conditions (21).

The World Health Organization (WHO) developed a standard classification for obesity based on body mass index (BMI) (defined as weight in kilograms divided by height in metres squared ( $\text{kg/m}^2$ )) and the risks associated with each classification of BMI and mortality, including type 2 diabetes and cardiovascular disease are shown on Table 1-1 (1). The WHO states that obesity is present when BMI is 30 or greater, however it does not differentiate between weight associated with fat mass and weight associated with lean body weight (muscle mass) (1).

**Table 1-1. WHO Classification of Obesity for Adults According to BMI<sup>a</sup>**

<b>Classification</b>	<b>BMI (kg/m<sup>2</sup>)</b>	<b>Risk of Comorbidities</b>
Underweight	< 18.50	Low (but risk of other clinical problems increased)
Normal range	18.50 – 24.99	Average
Overweight:	≥ 25.00	
Preobese	25.00 – 29.99	Increased
Obese class I	30.00 – 34.99	Moderate
Obese class II	35.00 – 39.99	Severe
Obese class III	≥ 40.00	Very Severe

<sup>a</sup> These BMI values are age-independent and the same for both sexes. However, BMI may not correspond to the same degree of fatness in different populations due, in part, to differences in body proportions. The table shows a simplistic relationship between BMI and the risk of comorbidity (disease), which can be affected by a range of factors, including the nature of the diet, ethnic group and activity level. The risks associated with increasing BMI are continuous and graded and begin at a BMI above 25. The interpretation of BMI gradings in relation to risk may differ for different populations. Both BMI and a measure of fat distribution (waist circumference or waist:hip ratio (WHR)) are important in calculating the risk of obesity comorbidities.

Two large population studies, the National Health and Nutrition Examination Surveys (NHANES) and the Study to Help Improve early evaluation and management of risk factors Leading to Diabetes (SHIELD) have shown a strong relationship between BMI and diabetes, hypertension and dyslipidemia ( $p < 0.001$ ) (23, 24). The NHANES also showed an association with obesity ( $\text{BMI} \geq 30$ ) and increased mortality (111,909 excess deaths; 95% CI, 53,754 – 170,064), and of the excess deaths associated with obesity, the majority of deaths occurred in individuals with a BMI of 35 or greater (82,066 deaths; 95% CI, 44,843 – 119,289) (23).

Other population studies have also shown that obesity was related to an increase in the development of cardiovascular disease. The Framingham Heart Study (5) showed the age-adjusted relative risk (RR), 95% CI for cardiovascular disease was increased in obese individuals ( $BMI \geq 30$ ) (men: 1.46 [1.20 – 1.77]; women: 1.64 [1.37 – 1.98]). Further, the Framingham Offspring Study (9) demonstrated that obesity ( $BMI \geq 30$ ) was predictive of the occurrence of first events in both coronary heart disease ( $p=0.05$ ) and cerebrovascular disease ( $p=0.03$ ).

## **2.1.2 Obesity and Body Fat Distribution**

### **2.1.2.1 Abdominal Obesity**

Abdominal obesity, characterized by the accumulation of visceral or intra-abdominal adipose tissue, is now recognized as an independent risk factor for the development of cardiovascular disease (CVD), type 2 diabetes, and metabolic syndrome (10, 11, 13, 25, 26, 27, 28, 30, 31, 32). The metabolic syndrome consists of metabolic disturbances such as insulin resistance, dysglycemia, dyslipidemia, and hypertension (25, 26, 27). Metabolic syndrome is strongly associated with abdominal obesity and is also recognized as a risk factor for developing CVD and type 2 diabetes (22, 25, 26, 28, 29, 31).

The National Cholesterol Education Program, Adult Treatment Panel III (NCEP, ATP III) acknowledges the WHO's definition of obesity according to BMI but also identifies obesity according to body fat distribution, specifically around the abdominal area (22). The NCEP ATP III states that abdominal obesity is more highly correlated with insulin resistance and metabolic syndrome than measures of BMI (22). The NCEP, ATP III identifies a waist circumference of greater than 102 cm in men, or greater than 88 cm in women as an indicator of abdominal obesity (22).

The NCEP, ATP III (22) defines the metabolic syndrome as having 3 out of 5 criteria on which clinicians can diagnose the syndrome, including a high waist circumference, high serum triglycerides, low high density lipoprotein cholesterol (HDL-C), high blood pressure, and high fasting glucose (Table 1-2).

**Table 1-2. NCEP-ATP III Clinical Identification of the Metabolic Syndrome**

<b>Risk Factor</b>	<b>Defining Level</b>
<b>Abdominal Obesity*</b>	Waist Circumference†
<b>Men</b>	>102 cm (40 inches)
<b>Women</b>	>88 cm (35 inches)
<b>Triglycerides</b>	≥1.7 mmol/L (150 mg/dL)
<b>HDL Cholesterol</b>	
<b>Men</b>	<1.0 mmol/L (<40 mg/dL)
<b>Women</b>	<1.3 mmol/L (<50 mg/dL)
<b>Blood Pressure</b>	≥130 / ≥85 mmHg
<b>Fasting Glucose</b>	≥6.1 mmol/L (110 mg/dL)

\* Overweight and obesity are associated with insulin resistance and metabolic syndrome. However, the presence of abdominal obesity is more highly correlated with the metabolic risk factors than is elevated body mass index (BMI). Therefore, the simple measure of waist circumference is recommended to identify the body weight component of the metabolic syndrome.

†Some male patients can develop multiple metabolic risk factors when the waist circumference is only marginally increased, eg. 94-102 cm (37-39 in). Such patients may have a strong genetic contribution to insulin resistance. They should benefit from changes in life habits, similarly to men with categorical increases in waist circumference.

### **2.1.3 Obesity and Insulin Resistance**

Insulin resistance, a key feature of the metabolic syndrome, is a complex, multi-organ dysfunction that is defined as an impairment of insulin action on glucose metabolism (33) or the inability of the cell to respond to the action of insulin. Insulin sensitivity on the other hand, is the degree to which a rise in plasma insulin can reduce postprandial blood glucose by stimulating glucose uptake in the peripheral tissues (skeletal muscle and adipose tissue) and suppress its production in the liver (33).

Insulin resistance rises with increases in body weight and body fat (26, 34). Most obese individuals ( $BMI \geq 30$ ) tend to have hyperinsulinemia and low insulin sensitivity (34), however it has been established that not all obese individuals are insulin resistant (34, 36). Imaging studies assessing abdominal adipose tissue (magnetic resonance imaging (MRI) and computed tomography (CT)) have shown that visceral adiposity is a strong predictor of insulin resistance in abdominally obese men (37), premenopausal women (38), and postmenopausal women (39) independent of subcutaneous abdominal and non-abdominal adipose tissue.

#### **2.1.3.1 Mechanisms Linking Obesity and Insulin Resistance**

Mechanisms have been proposed for the development of insulin resistance in those individuals with visceral adiposity. One proposed mechanism is that a combination of a sedentary lifestyle, high fat diet, and excess energy intake can result in a positive energy balance leading to adipocyte hypertrophy and an increase in visceral adipose tissue (36, 42, 43). This excess of energy intake results in an increase in circulating triglycerides (via portal circulation) and an increase in lipolysis, with a subsequent release of glycerol and free fatty acids (FFA) into circulation (40, 41). Within the adipose tissue, insulin promotes fatty acid re-esterification into triglyceride and inhibits lipolysis, therefore impairment of insulin action results in excess

circulating FFA (33). When the liver is exposed to elevated concentrations of FFA, it may lead to hyperinsulinemia (due to a decrease in insulin clearance), hyperglycemia (glucose intolerance due to an increase in hepatic glucose production), and dyslipidemia (low HDL-C, increased levels of low density lipoprotein cholesterol (LDL-C), high triglycerides, and an increase in very low density lipoprotein (VLDL) apolipoprotein B secretion) (40, 42).

A second proposed mechanism is that adipose tissue, especially visceral adiposity, is now recognized as an endocrine organ that secretes adipokines (cytokine-like molecules in adipose tissue including leptin, resistin, and adiponectin), inflammatory cytokines (such as interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )) and free fatty acids (FFA), which contribute to insulin resistance and the proinflammatory, prothrombotic, and hypertensive state (26, 40, 41, 42).

A third proposed mechanism is that individuals with abdominal obesity cannot store circulating lipids effectively in the subcutaneous adipose tissue (35, 36, 40). If this tissue is subjected to excessive amounts of lipids, there will be an increased delivery and redistribution of fats to organs other than adipose tissue such as the liver, pancreas, heart, and skeletal muscle (33, 35, 36, 40, 43). This notion has been referred to as lipotoxicity (35, 44) and suggests that increased lipids stored in these organs appear to inhibit insulin signaling, leading to a reduction in insulin-stimulated glucose uptake causing insulin resistance in these tissues (33, 35, 36, 40). This thesis will focus on the potential role of skeletal muscle lipid accumulation in insulin resistance.

## **2.2 Skeletal Muscle and Insulin Resistance**

Skeletal muscle facilitates insulin-stimulated glucose uptake and is the main contributor to glucose homeostasis in the body (33, 45). In obese individuals, elevated free fatty acids (FFA)

and excess fat located in the muscle cell appear to reduce insulin-stimulated glucose uptake and suppress muscle glycogen synthesis and glycolysis (33, 46) contributing to the development of skeletal muscle insulin resistance (41).

Fat stored in the muscle cell is categorized as either extramyocellular lipid (EMCL, lipids stored between muscle fibres) or intramyocellular lipid (IMCL, lipids stored within the muscle cell) (47). IMCL provides a readily available source of energy within the muscle exceeding that of glycogen stores (48, 49). However, abnormally high IMCL content tends to reduce the muscles sensitivity to insulin and therefore have become a focus of interest in the development of insulin resistance (48, 49, 50).

The impact of the accumulation of muscle triglyceride and the association with insulin resistance has been examined in muscle biopsies. In 1985, Falholt et al (51) first proposed the potential relationship between elevated muscle triglycerides and insulin resistance by reporting an increase in muscle triglycerides in normoglycemic, hyperinsulinemic dogs with low plasma triglycerides ( $171.4 \pm 46.6$  vs  $41.2 \pm 7.7 \mu\text{mol/g}$ ,  $p < 0.001$ ). In 1988, Falhot et al (52) then addressed the relationship between elevated muscle triglyceride in the development of insulin resistance in humans with type 2 diabetes. The diabetic patients showed an elevation of triglyceride in the muscle biopsies compared to the non-diabetic controls ( $290 \pm 52$  vs  $48 \pm 6 \mu\text{mol/g}$ ,  $p < 0.001$ ). Further proof linking elevated muscle triglycerides with insulin resistance came from later research demonstrating that muscle triglycerides are increased in healthy, normoglycemic women, varying in adiposity (50), young, healthy, overweight Pima Indians (54), and type I diabetics (53).

Computed tomography (CT) is a non-invasive approach to assess the distribution of adipose and lean tissue volume and density based on muscle attenuation values (Hounsfield



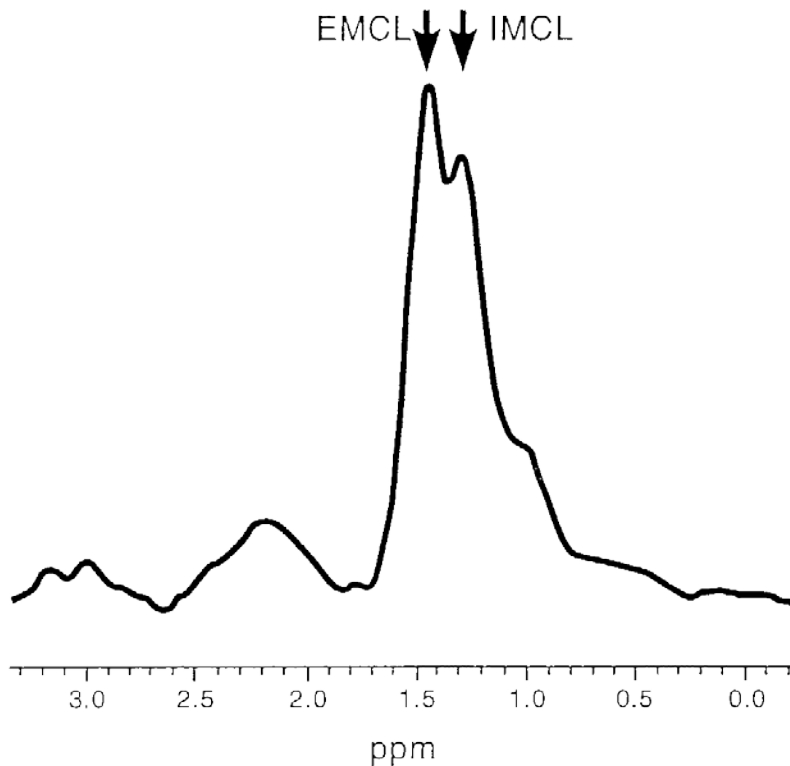
units), with lower attenuation values indicating a greater fat content within muscle (55). Simoneau et al (56) were first to report that muscle with reduced attenuation on CT scans (indicating elevated lipids in muscle) was strongly related to obesity and insulin resistance in healthy women. Later, Goodpaster et al observed a relationship between elevated muscle lipids (reduced muscle attenuation on CT) and insulin resistance in healthy sedentary, obese men and women (57) and in obese individuals with and without type 2 diabetics (58).

### **2.2.1 Proton-Nuclear Magnetic Resonance ( $^1\text{H}$ -NMR) Spectroscopy**

In the determination of skeletal muscle triglycerides, both muscle biopsies and CT techniques are unable to differentiate between IMCL and EMCL (47). Since research has shown that only IMCL is related to insulin resistance (59), it is necessary to quantify the intramyocellular triglyceride in order to establish the metabolic role of lipids within the muscle cell.

Proton-nuclear magnetic resonance ( $^1\text{H}$ -NMR) spectroscopy is a non-invasive imaging method that is able to distinguish between IMCL and EMCL muscle triglyceride content due to the different geometrical arrangement of the lipid compartments (47). IMCL are spherical droplets located within the cytoplasm of muscle cells close to the mitochondria, whereas EMCL are elongated droplets located between the muscle fibres (47). Chemical shift differences in IMCL and EMCL produce separate resonance frequencies which makes it possible to identify peaks in the spectral patterns that correspond to the methylene signals ( $\text{CH}_2$  resonances) of these two types of muscle lipids (47) (Figure 2-1 (63)). When the muscle fibre orientation is aligned with the axis of the magnet tube (Magnetic Resonance Imaging (MRI)), separation of the spectral patterns between the IMCL and EMCL is approximately 0.2 parts per million (ppm) (IMCL 1.28 ppm and EMCL 1.5 ppm on the spectrum, however there is slight variation for

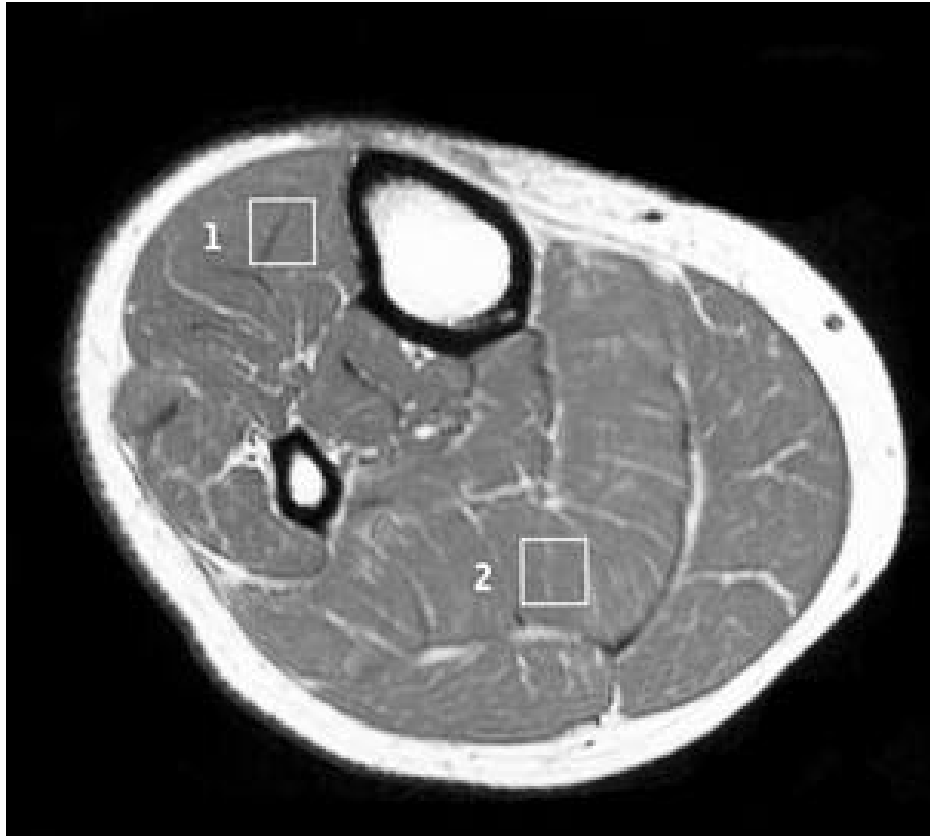
IMCL (ppm) and EMCL (ppm) between studies) (60, 61). Spectral intensities of IMCL and EMCL are referenced to either water or the methyl signal of Cr (Cr<sub>3</sub>, total creatine) (47, 60, 62).



**Figure 2.1. <sup>1</sup>H-NMR Spectra of the Human Soleus Muscle**

Methylene signals of IMCL and EMCL are indicated at 1.25 ppm and 1.4 ppm respectively (63).

Generally, the soleus or tibialis anterior calf muscle are the two most common muscles chosen in to identify IMCL using <sup>1</sup>H-NMR spectroscopy (Figure 2-2 (59)). The soleus muscle is highly oxidative due to the large amounts of slow twitch (Type I) muscle fibres, whereas the tibialis anterior muscle contains more fast twitch (Type II) glycolytic fibres and contain less IMCL than the soleus muscle (60). Both muscle groups have been used in <sup>1</sup>H-NMR spectroscopy to determine whether IMCL is a predictor of insulin resistance (59, 63, 64, 65, 66, 67, 68, 69).



**Figure 2.2. Magnetic Resonance Image of the Human Calf Muscle**

1 indicates the tibialis anterior muscle and 2 indicates the soleus muscle (59).

### **2.2.1.1 Intramyocellular lipid (IMCL) and Insulin Resistance/Insulin Sensitivity**

Studies using  $^1\text{H}$ -NMR spectroscopy in lean adults have shown a relationship between elevated IMCL and insulin resistance. Jacob et al (59) found that IMCL was 57% higher in the tibialis anterior muscle ( $3.26 \pm 0.36$  vs  $2.08 \pm 0.3$  arbitrary units,  $p < 0.017$ ) and 84% higher in the soleus muscle ( $11.8 \pm 1.6$  vs  $6.4 \pm 0.59$  arbitrary units,  $p = 0.008$ ) in lean insulin-resistant offspring adults of type 2 diabetic patients compared to matched insulin-sensitive offspring of type 2 diabetic patients. Jacob et al (59) also found a negative relationship between insulin sensitivity and the IMCL of the tibialis anterior muscle ( $r = -0.53$ ,  $p < 0.01$ ) and the soleus muscle ( $r = -0.35$ ,

but not significant  $p=0.1$ ), demonstrating that insulin resistance was correlated with IMCL. Perseghin et al (64) found similar results where offspring adults of type 2 diabetic parents were characterized with insulin resistance ( $p=0.04$ ) and increased IMCL content in the soleus muscle ( $p<0.01$ ). Perseghin et al (64) also found that IMCL of the soleus muscle was the main predictor of whole body insulin sensitivity ( $R^2=0.29$ ,  $p<0.01$ ). Krssak et al (63) also found an inverse correlation between IMCL and whole-body insulin sensitivity in a group of normal weight non-diabetic adults ( $r=-0.69$ ,  $p=0.0017$ ) using  $^1\text{H}$ -NMR spectroscopy of the soleus muscle.

Studies using  $^1\text{H}$ -NMR spectroscopy have also shown a relationship between elevated IMCL and insulin resistance in overweight and obese individuals. Sinha et al (65) found both the IMCL and EMCL content of the soleus muscle was significantly greater in obese adolescents compared to the lean, control adolescents ( $p<0.01$ ). Further, the results from Sinha et al (65) showed an inverse correlation between IMCL and insulin sensitivity ( $r=-0.59$ ,  $p<0.02$ ) and that this relationship was independent of percent total body fat and subcutaneous abdominal fat ( $r=-0.73$ ,  $p<0.01$ ) but not of visceral fat ( $r=-0.54$ ,  $p<0.08$ ). Moro et al (69) studied a group of sedentary obese non-diabetic and diabetic adults treated with sulfonylurea and metformin. In Moro's study (69), IMCL was inversely related to insulin sensitivity in both the soleus muscle ( $r=-0.48$ ,  $p=0.02$ ) and tibialis anterior muscle ( $r=-0.18$ , but not significant  $p=0.06$ ). Further, body fat was the main determinant of IMCL in the tibialis anterior muscle ( $r^2=0.30$ ,  $p=0.0054$ ) (69). In contrast to these studies, Perseghin et al (66) showed no significant differences in IMCL content in the soleus muscle ( $p=0.22$ ) or the tibialis anterior muscle ( $p=0.67$ ) in moderately overweight vs normal weight subjects. The lack of significance in IMCL in Perseghin's study (66) may be due to the small variation and definition of overweight according to BMI (overweight  $23.5\pm0.8$  vs normal weight  $21.2\pm0.6$   $\text{kg/m}^2$ ). Perseghin (66) did find that insulin

sensitivity was inversely related to the IMCL content of the soleus muscle ( $R^2=0.38$ ,  $p<0.01$ ) and the tibialis anterior muscle ( $R^2=0.36$ ,  $p<0.02$ ).

## **2.3 Effect of Diet on IMCL and Insulin Sensitivity**

### **2.3.1 High fat diets, IMCL and Insulin Sensitivity**

In animal studies, high fat feeding in rats increased muscle lipids and reported links between the accumulation of intramyocellular lipids and insulin resistance, characterized by reduced insulin-stimulated skeletal muscle glucose uptake and disposal (70, 71, 72, 73). In human studies using  $^1\text{H}$ -NMR spectroscopy to determine IMCL content, high fat diets have also been shown to increase IMCL in healthy individuals. Studies using a lipid infusion protocol to increase plasma FFA (74) or circulating non-esterified fatty acids (75) found increases in IMCL and corresponding changes in insulin sensitivity. Short term high fat diets of 3 days (75) and 7 days (76) consisting of approximately 60% total energy from dietary fat found that IMCL increased with high fat feeding as well as a decrease in insulin sensitivity (75). A longer crossover design study (25 days) consisting of a diet which was either low fat (30.8%), high fat (37.9%), or high in total fat (36.3%) and polyunsaturated fat (9.7%) in healthy adults with mildly elevated LDL-cholesterol found that IMCL was higher in the high fat diet but was not changed by type of dietary fat (77). St-Onge et al (77) did not find any correlations between IMCL and glucose or insulin concentrations.

### **2.3.2 Carbohydrate, IMCL and Insulin Sensitivity**

#### **2.3.2.1 Carbohydrate**

The majority of dietary carbohydrate consists of monosaccharides (glucose, fructose, galactose), disaccharides (sucrose, lactose, maltose), and polysaccharides (starch (amylose, amylopectin)), and fibre (soluble, insoluble)) (111, 112). Ingestion of dietary carbohydrate

increases postprandial blood glucose. This in turn results in the release of insulin by the pancreas to stimulate the uptake of glucose into the skeletal muscle and adipose tissue, as well as decreasing the production of glucose by the liver by reducing glycogenolysis and gluconeogenesis (111, 112). Postprandial blood glucose response is dependent on the type of dietary carbohydrate consumed. Foods with carbohydrate high in glucose are easily digested, absorbed rapidly, and have an immediate effect on postprandial blood glucose, whereas soluble fibre decreases the rate of glucose absorption and reduces the glycemic response (113). Specifically, soluble fibres high in viscosity (gel forming) slow carbohydrate absorption and decrease postprandial blood glucose responses (112). Plant starch occurs mainly as amylose and amylopectin however amylopectin breaks down more easily and digests more rapidly (112). Research has shown that consumption of high-amylose carbohydrates decreases the glucose and insulin responses in normal individuals (112, 114). Some starches are partially broken down and very slowly digested, or do not get digested at all in the human small intestine because humans lack the enzymes to break them down (115). These starches are termed partially resistant starch and resistant starch, and reduce the glycemic responses compared to readily digestible starch (115). Further, research has demonstrated the role of dietary fibre in reducing the risk for diseases including diabetes (116, 117) and cardiovascular disease (118). The interest in the glycemic effects of carbohydrate and the mechanisms by which dietary fibre improves blood glucose and insulin responses led to the development of the glycemic index (GI).

### **2.3.2.2 Glycemic Index**

The glycemic index (GI) was developed in 1981 by Jenkins et al (78) as a classification of the blood glucose-raising potential of the available carbohydrate in foods. The GI is defined as the 2 hour incremental area under the blood glucose response curve (iAUC, defined as the area

under the curve that is above fasting values only) after consuming a 50 gram available-carbohydrate (total carbohydrate minus fibre) portion of a test food expressed as a percentage of the response after consuming 50 grams of oral anhydrous glucose by the same subject (79, 80). The term glycemic load (GL) was also developed to account for the amount of carbohydrate in food and is calculated by multiplying the glycemic index of the food by the food's available carbohydrate (g) (81). Research has demonstrated that both the source and amount of carbohydrate in foods influence postprandial blood glucose and insulin (82, 83). Foods containing carbohydrate that are classified as low GI are digested and absorbed more slowly than high GI foods and would therefore have a lower postprandial blood glucose and insulin response due to the reduced rate of glucose absorption (81). Examples of carbohydrate foods with a low GI include barley, cooked pasta (al-dente), parboiled rice, beans, lentils, and oatmeal (non-instant), whereas white bread, cooked potatoes, and breakfast cereals (low in fibre) are a few examples of high GI foods (84). Since the GI is a classification of the degree to which carbohydrate containing foods increase blood glucose, foods containing solely protein and/or fat would not have a GI value (85). Further, differences in the amounts of fat and protein in normal meals have been shown to have little effect on the postprandial glucose and insulin responses (86).

Since its development, many studies with varying populations have shown that low GI diets improve fasting blood lipids (87, 88, 89, 90, 91, 92, 93, 94, 95, 96) and glycemic control (88, 92, 93, 94, 95, 96, 97, 98, 99), as well as decreasing the risk of developing cardiovascular disease (100), type 2 diabetes (101, 102, 103, 104), metabolic syndrome and insulin resistance, (105) and obesity (106), suggesting a possible role in the treatment and prevention of disease.

#### 2.3.2.2.1 Glycemic Index and Insulin Sensitivity

Research has demonstrated that low glycemic index carbohydrates can influence insulin sensitivity by improving glucose and insulin responses during the day as well as improving insulin-stimulated glucose uptake by adipocytes (93, 98, 99). Frost et al (98) studied the effects of a 4 week low-vs-high GI diet on glucose and insulin responses in patients with coronary heart disease as well as the relationship between insulin-stimulated glucose uptake in adipocytes (fat biopsy) and insulin sensitivity (hyperinsulinemic glucose clamp technique). A significant positive linear relationship was found between glucose uptake in adipocytes and the hyperinsulinemic glucose-clamp technique ( $r=0.72$ ,  $p<0.02$ ). Subjects on the low GI diet had a significant decrease in insulin iAUC ( $p<0.03$ ) and a significant increase in insulin-stimulated glucose uptake ( $p<0.05$ ). In a second study, Frost et al (99) studied the effects of a 3 week low-vs-high GI diet on *in-vivo* whole-body insulin sensitivity (measured by the short insulin tolerance test) and *in vitro* adipocyte insulin sensitivity (fat biopsy) in premenopausal women with a parental history of coronary heart disease. The low GI diet increased the *in vitro* adipocyte insulin sensitivity ( $p<0.05$ ) and the *in vivo* insulin sensitivity ( $p<0.01$ ). Although these two studies by Frost et al (98, 99) have demonstrated that low GI diets can improve insulin sensitivity, they have been criticized because the ‘gold standard’ euglycaemic, hyperinsulinaemic clamp technique was not used.

In a randomized, crossover study of either a low-vs-high glycemic index diet for 24 days in 20 type 2 diabetic subjects, Jarvi et al (93) evaluated insulin sensitivity using the euglycemic-hyperinsulinemic clamp technique. Subjects peripheral insulin sensitivity increased significantly on both the low GI diet ( $p<0.01$ ) and the high GI diet ( $p<0.05$ ), however it was more pronounced on the low GI diet compared to the high GI diet (30% vs 21% change respectively). The iAUC



was also 31% lower ( $p<0.05$ ) for blood glucose and 27% lower ( $p<0.01$ ) for plasma insulin following the low GI diet.

Rizkall et al (92) also performed a randomized crossover design study of a 4 week low and high glycemic index diet in 12 type 2 diabetic subjects using the euglycemic-hyperinsulinemic clamp technique to measure insulin sensitivity. Whole body glucose disposal was significantly higher following the low-vs-high GI diet ( $7.0\pm1.3$  vs  $4.8\pm0.9$ mg glucose/kg/min, respectively,  $p<0.001$ ). The iAUCs were also lower for plasma glucose after the low GI meals compared with the high GI meals during the 8 hour metabolic profiles at baseline (32%,  $p<0.05$ ) and at 4 weeks following the diet (47%,  $p<0.05$ ) and 23% lower for plasma insulin ( $p<0.05$ ) after the low GI diet compared to the high GI diet during the 8 hour metabolic profile at the beginning of the two dietary periods.

#### **2.3.2.2.2 Glycemic Index and IMCL**

Since low GI carbohydrates are slowly absorbed and release glucose gradually into the blood stream, they may suppress hepatic glucose production and non-esterified fatty acid (NEFA) release from adipocytes (107, 108). Reducing plasma fatty acids may decrease IMCL storage and promote insulin-stimulated glucose transport by muscle (109) creating a more insulin sensitive condition. Goff et al (110) investigated the effects of a 4 week low GI diet on insulin sensitivity (oral glucose tolerance test) and IMCL storage (magnetic resonance spectroscopy) in healthy adults. Results showed a significant improvement in the insulin sensitivity index (baseline  $7.8\pm1.1$  (SEM) vs post-intervention  $9.7\pm1.1$ ,  $p<0.02$ ) and a significant decrease in the insulin iAUC (baseline  $30.8\pm4.2$  vs post-intervention  $23.7\pm3.3$  mmol/min/L,  $p<0.01$ ). There were also significant decreases in fasting LDL cholesterol ( $p=0.01$ ), plasma triglycerides ( $p=0.01$ ), but no significant changes in NEFA concentrations ( $p=0.63$ ) post-intervention. IMCL

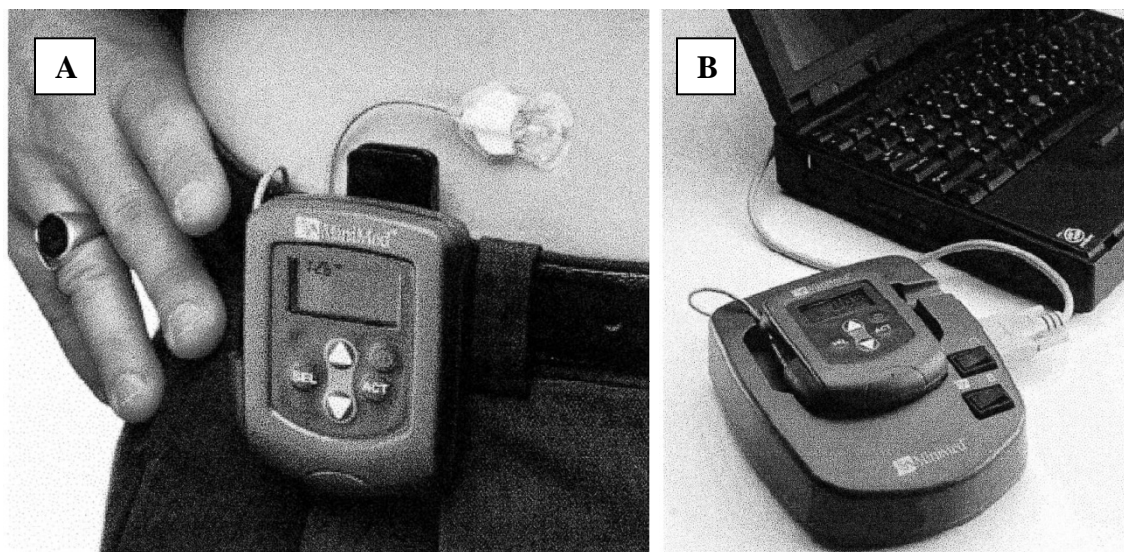
concentrations did not significantly change post-intervention, and no significant correlations were found between IMCL and insulin sensitivity suggesting that insulin sensitivity is independent of IMCL in healthy individuals. It must be noted however, that the reduction in GI was only 15% for four weeks, which may not have been enough of a reduction in GI or a long enough intervention to affect IMCL concentrations.

It still remains unclear whether a low GI diet can reduce IMCL stores in the muscles of individuals with abdominal obesity, a population at risk for developing insulin resistance and type 2 diabetes. The primary purpose of this thesis was to determine if a low GI diet would reduce IMCL stores thereby improving insulin sensitivity in individuals with abdominal obesity.

## **2.4 Continuous Glucose Monitoring System (CGMS)**

### **2.4.1 Overview of the CGMS**

Both fasting and postprandial blood glucose monitoring are used for the diagnosis and treatment of diabetes, as well as improving glycemic control and reducing the risk of complications arising from diabetes (119, 120). Self-monitoring blood glucose (SMBG) is an important practice for those individuals with diabetes (121), and may also help with glycemic control in non-diabetic individuals who are at risk for developing diabetes (e.g. obese individuals), however, there are limitations to this practice. Since blood glucose is influenced by changing variables throughout the day (e.g. stress, physical activity, diet, rate of nutrient absorption) (122)), SMBG would need to be performed several times throughout the day which may not be practical for most individuals. Continuous glucose monitoring systems were originally developed for diabetics to accurately identify blood glucose levels throughout the day (123). The MiniMed Continuous Glucose Monitoring System (CGMS; MiniMed Inc., Northridge, CA) (Figure 2.3) was the first to develop a continuous sensor that could monitor



**Figure 2.3. Continuous Glucose Monitoring System (MiniMed Inc, Northridge, CA)**

A: CGMS Monitor and Sensor; B: CGMS Com-Station (123)

glucose profiles for 72 hours (123) and is the monitor used in this research (refer to Chapter 6). This model is a Holter-style sensor system consisting of the following components: a glucose monitor that stores the electrical signals from the glucose sensor; a sensor inserter (senserter) which is used to insert the sensor; a sterile, subcutaneous glucose sensor that continuously detects electrical signals (measured in nanoamperes (nA)) every 10 seconds by the reaction of glucose in the interstitial fluid with glucose oxidase, and averages 30 electrical signals every 5 minutes from the sensor for a total of 288 measurements per day; a connecting cable; and a communication device (Com-Station) which enables glucose data stored in the monitor to be downloaded to a computer to be analyzed (123). Once the sensor is inserted, typically in the abdominal area, the individual must enter at least four blood glucose values obtained from a portable finger-stick glucose meter to calibrate the monitor (123). Since the original development of the CGMS, MiniMed Inc. and other manufacturers have updated the design of the monitors to give the individual real-time access to their blood glucose values, as well as

providing alarms when blood glucose values reach hypoglycemic and hyperglycemic ranges (124, 125). Research has shown that CGMS is beneficial for glycemic control in patients with diabetes and is an important clinical tool to help health care providers make recommendations to patients for reducing complications arising from diabetes (124, 126, 127, 128).

#### **2.4.2 CGMS and Glycemic Index**

Research has examined the effects of glycemic index on glycemic control using the CGMS in type 2 diabetics (129), type 1 diabetics (130), healthy adults (131), and healthy adults at risk for heart disease (132). Brynes et al (129) investigated the effects of a 7 day low GI diet (LGID) on glycemic profiles in free-living type 2 diabetics using the CGMS for two 24 hour periods. Significant reductions were found in fasting glucose ( $p<0.01$ ), 24 hour area under the curve (AUC) for glucose ( $p<0.04$ ), and overnight 8 hour AUC glucose ( $p<0.05$ ), suggesting that glycemic control can be improved following a short term LGID (129). Byrnes et al (131) performed another study in free-living healthy young adults, and used the CGMS to investigate changes in blood glucose profiles after following a 7 day LGID. A significant reduction was found in fasting blood glucose ( $p<0.001$ ), mean blood glucose over the 24 hour period ( $p=0.004$ ), area under the 24 hour glucose curve ( $p=0.004$ ), and overnight 8 hour glucose curve ( $p=0.01$ ), suggesting that a LGID can have a significant effect on glucose profiles by improving hepatic insulin sensitivity and decreasing hepatic glucose output (131). Nansel et al (130) used the CGMS to examine changes in glycemic profiles in type 1 diabetic youths, following a one day high vs low GI diet in a controlled setting, cross-over study. Participants demonstrated lower daytime mean blood glucose and lower blood glucose area under the curve ( $p<0.001$ ) following the low GI diet, suggesting that a low GI diet may reduce glucose excursions and improve glycemic control (130). Philippou et al (132) studied the effects of a 12 week LGID on

24 hour glycemic responses using the CGMS in free-living adults at risk for developing heart disease. Results showed a significantly lower 24 hour area under the curve (AUC) for glucose ( $p=0.045$ ) and overnight AUC ( $p=0.006$ ) for the LGID compared to the high GI diet but no significant changes in blood lipids ( $p>0.05$ ) (132). Further, Philippou et al (132) found that both groups significantly reduced their energy intake ( $p<0.05$ ) but only the LGID group lost weight, concluding that a LGID in addition to weight loss may reduce cardiovascular risk. These studies (129, 130, 131, 132) showed that the GI can reduce fasting and postprandial blood glucose responses in individuals who are given specific foods to consume, however, the question about whether the GI can predict individual glycemic responses to self-selected meals remains unclear. Fabricatore et al (133) addressed this issue by examining the relationship of the GI and other dietary variables to glycemic responses, assessed by a CGMS, when meals were consumed in self-selected amounts by free-living overweight and obese type 2 diabetics. Results showed that GI was positively related to CGMS AUC glucose ( $p=0.01$ ), mean glucose ( $p=0.01$ ), and time spent in a hyperglycemic range of greater than 10 mmol/L ( $p=0.02$ ), and a multiple regression analysis showed that the dietary GI was the strongest predictor of glycemic variability accounting for 10% to 18% of the variance in each glycemic variable, independent of energy and carbohydrate intake (133). Fabricatore et al (133) concluded that the results of their study support the validity of the GI and that consumption of a LGID is beneficial for controlling blood glucose in type 2 diabetics. A purpose of this thesis was to determine whether GI is a significant determinant of individual glycemic responses elicited by self-selected breakfast meals in healthy, abdominally obese adults, a population at risk for diabetes.

## 2.5 References

1. World Health Organization. Obesity: preventing and managing the global epidemic: report of a WHO consultation. WHO Technical Report Series No. 894. 2000. Geneva, Switzerland: World Health Organization.
2. Health Canada. Map of obesity according to measured body mass index (BMI) in adults in Canada (both males and females). *Canadian Community Health Survey Cycle 2.2, Nutrition*, 2004.
3. James PT, Leach R, Kalamara E, Shayeghi M. The worldwide obesity epidemic. *Obesity Research* 2001;9:228S–233S.
4. Scuro LA, Bosello O. The problem of classifying obesity. *World Journal of Obesity* 1981;5:789–794.
5. Wilson PW, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Archives of Internal Medicine* 2002;162:1867-1872.
6. Kurth T, Gaziano JM, Berger K, Kase CS, Rexrode KM, Cook NR, Buring JE, Manson JE. Body mass index and the risk of stroke in men. *Archives of Internal Medicine* 2002;162:2557-2562.
7. Rexrode KM, Hennekens CH, Willett WC, Colditz GA, Stampfer MJ, Rech-Edwards JW, Speizer FE, Manson JE. A prospective study of body mass index, weight change, and risk stroke in women. *Journal of the American Medical Association* 1997;277:1539-1545.

8. Brown CD, Higgins M, Donato KA, Rohde FC, Garrison R, Obarzanek E, Ernst ND, Horan M. Body mass index and the prevalence of hypertension and dyslipidemia. *Obesity Research* 2000;8:605-619.
9. Wilson PWF, Bozeman SR, Burton TM, Hoaglin DC, Ben-Joseph R, Pashos CL. Prediction of first events of coronary heart disease and stroke with consideration of adiposity. *Circulation* 2008;118:124-130.
10. Bray GA, Jablonski KA, Fujimoto WY, Barrett-Connor E, Haffner S, Hanson RL, Hill JO, Hubbard V, Kriska A, Stamm E, Pi-Sunyer FX. Relation of central adiposity and body mass index to the development of diabetes in the Diabetes Prevention Program. *American Journal of Clinical Nutrition* 2008;87:1212-1218.
11. Carey VJ, Walters EE, Colditz GA, Solomon CG, Willett WC, Rosner BA, Speizer FE, Manson JE. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. *American Journal of Epidemiology* 1997;145:614-619.
12. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 1994;17:961-969.
13. Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. *American Journal of Clinical Nutrition* 2005;81:555-563.
14. Peppard PE, Young T, Palta M, Dempsey J, Skatrud J. Longitudinal Study of Moderate Weight Change and Sleep-Disordered Breathing. *Journal of the American Medical Association* 2000;284:3015-3021.

15. Griffin KA, Kramer H, Bidani AK. Adverse renal consequences of obesity. *American Journal of Physiology Renal Physiology* 2008;294:F685-F696.
16. Thygesen LC, Gronbaek M, Johansen C, Fuchs CS, Willett WC, Giovannucci E. Prospective weight change and colon cancer risk in male US health professionals. *International Journal of Cancer* 2008;123:1160-1165.
17. Olsen CM, Nagle CM, Whiteman DC, Purdie DM, Green AC, Webb PM, Australian Cancer Study (Ovarian Cancer), Australian Ovarian Cancer Study Group. Body size and risk of epithelial ovarian and related cancers: A population-based case-control study. *International Journal of Cancer* 2008;123:450-456.
18. Han D, Nie J, Bonner MR, McCann SE, Muti P, Trevisan M, Ramirez-Marrero FA, Vito D, Freudenheim JL. Lifetime adult weight gain, central adiposity, and the risk of pre- and postmenopausal breast cancer in the Western New York exposures and breast cancer study. *International Journal of Cancer* 2006;119:2931-2937.
19. Chang S, Lacey JV, Brinton LA, Hartge P, Adams K, Mouw T, Carroll L, Hollenbeck A, Schatzkin A, Leitzmann MF. Lifetime weight history and endometrial cancer risk by type of menopausal hormone use in the NIH-AARP diet and health study. *Cancer Epidemiology Biomarkers & Previews* 2007;16:723-730.
20. Dittrick GW, Thompson JS, Campos D, Bremers D, Sudan D. Gallbladder pathology in morbid obesity. *Obesity Surgery* 2005;15:238-242.
21. Anandacoomarasamy A, Caterson I, Sambrook P, Fransen M, March L. The impact of obesity on the musculoskeletal system. *International Journal of Obesity* 2008;32:211-222.



22. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment on high blood cholesterol in adults (Adult Treatment Panel III). *Journal of the American Medical Association* 2001;285:2486-2497.
23. Flegal KM, Graubard BI, Williamson DF, Gail MH. Excess deaths associated with underweight, overweight, and obesity. *Journal of the American Medical Association* 2005;293:1861-1867.
24. Bays HE, Chapman RH, Grandy S. (for the SHIELD Investigators' Group). The relationship of body mass index to diabetes mellitus, hypertension and dyslipidaemia: comparison of data from two national surveys. *International Journal of Clinical Practice* 2007;61:737-747.
25. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991;14:173-194.
26. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004;109:433-438.
27. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006;444:881-887.
28. Lois K, Young J, Kumar S. Obesity; epiphenomenon or cause of metabolic syndrome? *International Journal of Clinical Practice* 2008;62:932-938.

29. Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. *American Journal of Medicine* 2006;119:812-819.
30. Freemantle N, Holmes J, Hockey A, Kumar S. How strong is the association between abdominal obesity and the incidence of type 2 diabetes? *International Journal of Clinical Practice* 2008;62:1391-1396.
31. Gastaldelli A. Abdominal fat: does it predict the development of type 2 diabetes? *American Journal of Clinical Nutrition* 2008;87:1118-1119.
32. Balkau B, Deanfield JE, Despres JP, Bassand JP, Fox, KAA, Smith SC Barter P, Tan C, Van Gaal L, Wittchen HU, Massien C, Haffner SM. International day for the evaluation of abdominal obesity (IDEA): A study of waist circumference, cardiovascular disease, and diabetes mellitus in 168 000 primary care patients in 63 countries. *Circulation* 2007;116:1942-1951.
33. Gastaldelli A, Natali A, Vettor R, Corradini SG. Insulin resistance, adipose depots and gut: Interactions and pathological implications. *Digestive and Liver Disease* 2010;42:310-319.
34. Abassi F, Brown BW, Lamendola C, McLaughlin T, Reaven GM. Relationship between obesity, insulin resistance, and coronary heart disease risk. *Journal of the American College of Cardiology* 2002;40:937-943.
35. McGarry JD, Dobbins RL. Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 1999;42:128-138.
36. Shulman GI. Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy. *Physiology* 2004;19:183-190.

37. Ross R, Aru J, Freeman J, Hudson R, Janssen I. Abdominal adiposity and insulin resistance in obese men. *American Journal of Physiology, Endocrinology, and Metabolism* 2002;282:E657-E663.
38. Ross R, Freeman, Hudson R, Janssen I. Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. *Journal of Clinical Endocrinology & Metabolism* 2002;87:5044-5051.
39. Brochu M, Starling RD, Tchernof A, Matthews DE, Garcia-Rubi E, Poehlman ET. Visceral adipose tissue is an independent correlate of glucose disposal in older obese postmenopausal women. *Journal of Clinical Endocrinology & Metabolism* 2000;85:2378-2384.
40. Despres JP, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E, Rodes-Cabau J, Bertrand OF, Poirier P. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2008;28:1039-1049.
41. Benito M. Tissue specificity on insulin action and resistance: past to recent mechanisms. *Acta Physiologica* 2011;201:297-312.
42. Potenza MV, Mechanick JI. The metabolic syndrome: definition, global impact, and pathophysiology. *Nutrition in Clinical Practice* 2009;24:560-577.
43. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006;444:881-887.
44. Unger RH. The physiology of cellular liporegulation. *Annual Review of Physiology* 2003;65:333-347.

45. Stefanyk LE, Dyck DJ. The interaction between adipokines, diet and exercise on muscle insulin sensitivity. *Current Opinion in Clinical Nutrition and Metabolic Care* 2010;13:255-259.
46. Roden M, Price TB, Perseghin G, Flk Pertersen K, Rothman DL, Cline GW, Shulman G. Mechanism of free fatty acid-induced insulin resistance in humans. *Journal of Clinical Investigation* 1996;97:2859-2865.
47. Machann J, Haring H, Schick F, Stumvoll M. Intramyocellular lipids and insulin resistance. *Diabetes, Obesity and Metabolism* 2004;6:239-248.
48. Komiya H, Mori Y, Yokose T, Kurokawa N, Horie N, Tajima N. Effect of intramuscular fat difference on glucose and insulin reaction in oral glucose tolerance test. *Journal of Atherosclerosis and Thrombosis* 2006;13:136-142.
49. Oscai LB, Essig DA, Palmer WK. Lipase regulation of muscle triglyceride hydrolysis. *Journal of Applied Physiology* 1990;69:1571-1577.
50. Phillips DIW, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwic AC, Taylor R. Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism* 1996;45:947-950.
51. Falholt K, Cutfield R, Alejandro R, Heding L, Mintz D. The effects of hyperinsulinemia on arterial wall and peripheral muscle metabolism in dogs. *Metabolism* 1985;34:1146-1149.
52. Falholt K, Jensen I, Lindkaer Jensen S, Mortensen H, Volund AA, Heding LG, Noerskov Petersen P, Falholt W. Carbohydrate and lipid metabolism of skeletal muscle in Type 2 diabetic patients. *Diabetic Medicine* 1988;5:27-31.

53. Ebeling P, Essen-Gustavsson B, Tuominen JA, Koivisto VA. Intramuscular triglyceride content is increased in IDDM. *Diabetologia* 1998;41:111-115.
54. Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 1997;46:983-988.
55. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *Journal of Applied Physiology* 2000;89:104-110.
56. Simoneau J, Colberg SR, Thaete FL, Kelley DE. Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. *Federation of American Societies for Experimental Biology*. 1995;9:273-278.
57. Goodpaster BH, Thaete FL, Simoneau J, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes* 1997;46:1579-1585.
58. Goodpaster BH, Thaete FL, Kelley DE. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. *American Journal of Clinical Nutrition* 2000;71:885-892.
59. Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, Haring HU. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 1999;48:1113-1119.

60. Hwang JH, Pan JW, Heydari S, Hetherington HP, Stein DT. Regional differences in intramyocellular lipids in humans observed by in vivo  $^1\text{H}$ -MR spectroscopic imaging. *Journal of Applied Physiology* 2001;90:1267-1274.
61. Schick F, Eismann B, Jung WI, Bongers H, Bunse M, Lutz O. Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: Two lipid compartments in muscle tissue. *Magnetic Resonance in Medicine* 1993;29:158-167.
62. Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT. Measurement of intracellular triglyceride stores by  $^1\text{H}$  spectroscopy: validation in vivo. *American Journal of Physiology* 1999;276:E977-E989.
63. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Shulman GI, Roden M. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a  $^1\text{H}$  NMR spectroscopy study. *Diabetologia* 1999;42:113-116.
64. Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans. A  $^1\text{H}$ - $^{13}\text{C}$  nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 1999;48:1600-1606.
65. Sinha R, Dufour S, Falk Peterson, K, LeBon V, Enoksson S, Ma Y, Savoye M, Rothman DL, Shulman GI, Caprio S. Assessment of skeletal muscle triglyceride content by  $^1\text{H}$  nuclear magnetic resonance spectroscopy in lean and obese adolescents. Relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes* 2002;51:1022-1027.
66. Perseghin G, Scifo P, Danna M, Battezzati A, Benedini S, Meneghini E, Del Maschio A, Luzi L. Normal insulin sensitivity and IMCL content in overweight humans are

associated with higher fasting lipid oxidation. *American Journal of Physiology, Endocrinology, and Metabolism* 2002;283:E556-E564

67. Misra A, Sinha S, Kumar M, Jagannathan NR, Pandey RM. Proton magnetic resonance spectroscopy study of soleus muscle in non-obese healthy and Type 2 diabetic Asian Northern Indian males: high intramyocellular lipid content correlates with excess body fat and abdominal obesity. *Diabetic Medicine* 2003;20:361-367.
68. Bredella MA, Torriani M, Ghomi RH, Thomas BJ, Brick DJ, Gerweck AV, Harrington LM, Miller KK. Adiponectin is inversely associated with intramyocellular and intrahepatic lipids in obese premenopausal women. *Obesity* 2011;19:911-916.
69. Moro C, Galgani JE, Luu L, Pasarica M, Mairal A, Bajpeyi S, Schmitz G, Langin D, Liebisch G, Smith SR. Influence of gender, obesity, and muscle lipase activity on intramyocellular lipids in sedentary individuals. *Journal of Clinical Endocrinology and Metabolism* 2009;94:3440-3447.
70. Storlien LH, James DE, Burleigh KM, Chisholm DJ, Kraegen EW. Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. *American Journal of Physiology, Endocrinology & Metabolism* 1986;14:E576-E583.
71. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes* 1991;40:280-289.
72. Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH. Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes* 1991;40:1397-1407.

73. Oakes ND, Cooney GJ, Camilleri S, Chisholm DJ, Kraegen EW. Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. *Diabetes* 1997;46:1768-1774.
74. Boden G, Lebed B, Schatz M, Homko C, Lemieux S. Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes* 2001;50:1612-1617.
75. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, Loviscach M, Stumvoll M, Claussen CD, Schick F, Haring HU, Jacob S. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 2001;50:2579-2584.
76. Schrauwen-Hinderling VB, Kooi ME, Hesselink MKC, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie G, Saris WHM, Nicolay K, Schrauwen P. Intramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet. *Obesity Research* 2005;13:2088-2094.
77. St-Onge MP, Newcomer BR, Buchthal S, Aban I, Allison DB, Bosarge A, Gower B. Intramyocellular lipid content is lower with a low-fat diet than with high-fat diets, but that may not be relevant for health. *American Journal of Clinical Nutrition* 2007;86:1316-1322.
78. Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 1981;34:362-366.
79. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. *American Journal of Clinical Nutrition* 1991;54:846-854.



80. Wolever TM. Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values. *British Journal of Nutrition* 2004;91:295-300.
81. Jenkins DJA, Kendall CWC, Augustin LSA, Franceschi S, Hamidi M, Marchie A, Jenkins AL, Axelsen M. Glycemic index: overview of implications in health and disease. *American Journal of Clinical Nutrition* 2002;76(suppl):266S-273S.
82. Wolever TMS, Bolognesi C. Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. *Journal of Nutrition* 1996;126:2798-2806.
83. Lee BM, Wolever TMS. Effect of glucose, sucrose and fructose on plasma glucose and insulin responses in normal humans: comparison with white bread. *European Journal of Clinical Nutrition* 1998;52:924-928.
84. Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. *American Journal of Clinical Nutrition* 2002;76:5-56.
85. Wolever TMS. The glycemic index. A physiological classification of dietary carbohydrate. Oxfordshire, OX, UK: CABI Publishing; 2006.
86. Wolever TMS, Bolognesi C. Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index. *Journal of Nutrition* 1996;126:2807-2812.
87. Jenkins DJA, Wolever TMS, Kalmusky J, Guidici S, Giordano C, Patten R, Wong GS, Bird JN, Hall M, Buckley G, Csimas A, Little JA. Low-glycemic index diet in hyperlipidemia: use of traditional starchy foods. *American Journal of Clinical Nutrition* 1987;46:66-71.

88. Jenkins DJA, Wolever TMS, Collier GR, Ocana A, Rao, AV, Buckley G, Lam Y, Mayer A, Thompson LU. Metabolic effects of a low-glycemic-index diet. *American Journal of Clinical Nutrition* 1987;46:968-975.
89. Sloth B, Drog-Mikkelsen K, Flint A, Tetens I, Bjorck I, Vinoy S, Elmstahl H, Astrup A, Lang V, Raben A. No difference in body weight decrease between a low-glycemic-index and a high-glycemic-index diet but reduced LDL cholesterol after 10-wk ad libitum intake of the low-glycemic-index diet. *American Journal of Clinical Nutrition* 2004;80:337-347.
90. Frost G, Leeds AA, Dore CJ, Madeiros S, Brading S, Dornhorst. Glycaemic index as a determinant of serum HDL-cholesterol concentration. *Lancet* 1999;353:1045-1048.
91. Ford ES, Liu S. Glycemic index and serum high-density lipoprotein cholesterol concentration among US adults. *Archives of Internal Medicine* 2001;161:572-576.
92. Rizkalla SW, Taghrid L, Laromiguire M, Huet D, Boillot J, Rigoir A, Elgrably F, Slama G. Improved plasma glucose control, whole-body glucose utilization, and lipid profile on a low-glycemic index diet in type 2 diabetic men. *Diabetes Care* 2004;27:1866-1872.
93. Jarvi AE, Karlstrom BE, Granfeldt YE, Bjorck IE, Asp NL, Vessby EOH. Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients. *Diabetes Care* 1999;22:10-18.
94. Fontvieille AM, Rizkalla SW, Penfornis A, Acosta M, Bornet FR, Slama G. The use of low glycaemic index foods improves metabolic control of diabetic patients over five weeks. *Diabetic Medicine* 1992;9:444-450.

95. Wolever TMS, Jenkins DJ, Vuksan V, Jenkins AL, Wong GS, Josse RG. Beneficial effect of low-glycemic index diet in overweight NIDDM subjects. *Diabetes Care* 1992;15:562-564.
96. Wolever TMS, Jenkins DJ, Vuksan V, Jenkins AL, Buckley GC, Wong GS, Josse RG. Beneficial effect of a low-glycaemic index diet in type 2 diabetes. *Diabetic Medicine* 1992;9:451-458.
97. Brand-Miller J, Hayne S, Petocz P, Colagiuri S. Low-glycemic index diets in the management of diabetes. *Diabetes Care* 2003;26:2261-2267.
98. Frost G, Keogh B, Smith D, Akinsanya K, Leeds A. The effect of low-glycemic carbohydrate on insulin and glucose response in vivo and in vitro in patients with coronary heart disease. *Metabolism* 1996;45:669-672.
99. Frost G, Leeds A, Trew G, Margara R, Dornhorst A. Insulin sensitivity in women at risk of coronary heart disease and the effect of a low glycemic diet. *Metabolism* 1998;47:1245-1251.
100. Liu S, Willett W, Stampfer MJ, Hu FB, Franz M, Sampson L, Hennekens CH, Manson JE. A prospective study of dietary glycemic load, carbohydrate intake and risk of coronary heart disease in US women. *American Journal of Clinical Nutrition* 2000;71:1455-1461.
101. Salmeron J, Manson JJ, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk on non-insulin-dependent diabetes mellitus in women. *Journal of the American Medical Association* 1997;277:472-477.

102. Salmeron J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ, Stampfer MJ, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care* 1997;20:545-550.
103. Schulze MB, Liu S, Rimm EB, Manson JE, Willett WC, Hu FB. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. *American Journal of Clinical Nutrition* 2004;80:348-356.
104. Hodge AM, English DR, O'Dea K, Giles GG. Glycemic index and dietary fiber and the risk of type 2 diabetes. *Diabetes Care* 2004;27:2701-2706.
105. McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PWF, Jacques PF. Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham offspring cohort. *Diabetes Care* 2004;27:538-546.
106. Brand-Miller JC, Holt SHA, Pawlak DB, McMillan J. Glycemic index and obesity. *American Journal of Clinical Nutrition* 2002;76(suppl):281S-285S.
107. Wolever TMS, Bentum-Williams A, Jenkins DJA. Physiological modulation of plasma free fatty acid concentrations by diet: Metabolic implications in nondiabetic subjects. *Diabetes Care* 1995;18:962-970.
108. Thorburn A, Muir J, Proietto J. Carbohydrate fermentation decreases hepatic glucose output in healthy subject. *Metabolism* 1993;42:780-785.
109. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unraveling the mechanism. *Lancet* 2010;375:2267-2277.
110. Goff LM, Frost GS, Hamilton G, Thomas EL, Dhillon WS, Dornhorst A, Bell JD. Carbohydrate-induced manipulation of insulin sensitivity independently of intramyocellular lipids. *British Journal of Nutrition* 2003;89:365-374.

111. Bessesen DH. The role of carbohydrate in insulin resistance. *Journal of Nutrition* 2001;131:2782S-2786S.
112. Morris KL, Zemel MB. Glycemic index, cardiovascular disease, and obesity. *Nutrition Reviews* 1999;57:273-276.
113. Englyst KN, Englyst HN. Carbohydrate bioavailability. *British Journal of Nutrition* 2005;94:1-11.
114. Behall KM, How JC. Effect of long-term consumption of amylase vs amylopectin starch on metabolic variables in human subjects. *American Journal of Clinical Nutrition* 1995;61:334-340.
115. Englyst HN, Cummings JH. Digestion of polysaccharides of potato in the small intestine of man. *American Journal of Clinical Nutrition* 1987;45:423-431.
116. Salmeron J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ, Stampfer MJ, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care* 1997;20:545-550.
117. Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load and risk on non-insulin-dependent diabetes mellitus in women. *Journal of the American Medical Association* 1997;277:472-477.
118. Lupton JR, Turner ND. Dietary fiber and coronary disease: does the evidence support an association? *Current Atherosclerosis Reports* 2003;5:500-505.
119. Canadian Diabetes Association. 2008 clinical practice guidelines for the prevention and management of diabetes in Canada. *Canadian Journal of Diabetes* 2008;32:S10-S13.

120. Coutinho M, Gerstein HC, Wang Y, Yusef S. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care* 1999;22:233-240.
121. American Diabetes Association. Self-monitoring of blood glucose (consensus statement). *Diabetes Care* 1994;17:81-86.
122. Hirsch IB, Armstrong D, Bergenstal RM, Buckingham B, Childs BP, Clarke WL, Peters A, Wolpert H. Clinical application of emerging sensor technologies in diabetes management: Consensus guidelines for continuous glucose monitoring (CGM). *Diabetes Technology and Therapeutics* 2008;10:232-246.
123. Mastrototaro JJ. The MiniMed continuous glucose monitoring system. *Diabetes Technology and Therapeutics* 2000;2:S13-S22.
124. Reach G. Continuous glucose monitoring and diabetes health outcomes: a critical appraisal. *Diabetes technology and Therapeutics* 2008;10:69-80.
125. Wentholt IME, Hart AAM, Hoekstra JBL, DeVries JH. How to assess and compare the accuracy of continuous glucose monitors? *Diabetes Technology and Therapeutics* 2008;10:57-68.
126. Gross TM, Bode BW, Einhorn D, Kayne DM, Reed JH, White NH, Mastrototaro JJ. Performance evaluation of the MiniMed continuous glucose monitoring system during patient home use. *Diabetes Technology and Therapeutics* 2000;2:49-56.
127. Hermanides J, DeVries JH. Sense and nonsense in sensors. *Diabetologia* 2010;53:593-596.

128. Joubert M, Reznik Y. Personal continuous glucose monitoring (CGM) in diabetes management: Review of the literature and implementation for practical use. *Diabetes Research and Clinical Practice* 2012;96:294-305.
129. Brynes AE, Lee JL, Brighton RE, Leeds AR, Ornhorst A, Frost GS. A low glycemic diet significantly improves the 24-h blood glucose profile in people with type 2 diabetes, as assessed using the continuous glucose MiniMed monitor. *Diabetes Care* 2003;26:548-549.
130. Nansel TR, Gellar, McGill A. Effect of varying glycemic index meals on blood glucose control assessed with continuous glucose monitoring in youth with type 1 diabetes on basal-bolus insulin regimens. *Diabetes Care* 2008;31:695-697.
131. Brynes AE, Adamson J, Dornhorst A, Frost GS. The beneficial effect of a diet with low glycaemic index on 24h glucose profiles in healthy young people as assessed by continuous glucose monitoring. *British Journal of Nutrition* 2005;93:179-182.
132. Philippou E, McGowan BMC, Brynes AE, Dornhorst A, Leeds AR, Frost GS. The effect of a 12-week low glycaemic index diet on heart disease risk factors and 24h glycaemic response in healthy middle-aged volunteers at risk of heart disease: a pilot study. *European Journal of Clinical Nutrition* 2008;62:145-149.
133. Fabricatore AN, Ebbeling CB, Wadden TA, Ludwig DS. Continuous glucose monitoring to assess the ecologic validity of dietary glycemic index and glycemic load. *American Journal of Clinical Nutrition* 2011;94:1519-1524.

**CHAPTER 3**  
**RATIONALE, HYPOTHESES, AND OBJECTIVES**



### **3. Rationale, Hypotheses, and Objectives**

Research has shown that insulin sensitivity may in part be determined by the increased storage of lipids in organs other than adipose tissue such as muscle. It has been hypothesized that increased lipid storage in muscle reduces sensitivity to insulin, thereby reducing insulin-mediated glucose uptake in muscle. This would lead to elevated insulin and glucose levels which in turn may lead to insulin resistance. Although research has shown a relationship between insulin sensitivity and IMCL, it still remains unclear whether IMCL stores are a cause or an effect of insulin resistance.

Although controversial, the role of the glycemic index (GI) in influencing insulin sensitivity has been documented. It has been hypothesized that low GI diets may improve insulin sensitivity by suppressing non-esterified fatty acid release. This in turn may reduce IMCL storage by promoting skeletal muscle glucose uptake.

It has been suggested that individuals with abdominal obesity have elevated levels of IMCL. Documenting a positive effect of a low glycemic index diet on IMCL content and insulin sensitivity may have important implications in the treatment for individuals with abdominal obesity, as well as potentially help in the prevention of metabolic and cardiovascular complications in these individuals. Further, demonstrating that a low GI diet as an intervention can reduce IMCL content and improve insulin sensitivity would be considered a major step forward in the understanding of insulin resistance.

This dissertation will be divided into three research chapters to address the information discussed above, each with their hypotheses and objectives. All three chapters are from one long-term study. The first chapter is the low-fat dietary advice run-in phase of the main study, the second chapter is the main study, and the third chapter is a sub-study from the run-in phase.

### **3.1 Effects of an ad-libitum low fat diet on metabolic profiles in adults with abdominal obesity**

#### **Hypothesis:**

Following dietary advice on lowering overall fat and saturated fat intake will improve metabolic profiles in adults with abdominal obesity.

#### **Objective:**

The primary purpose of designing a 4 to 6 week low fat dietary advice run-in phase prior to the main study was to eliminate potential effects of variations in dietary fat intake which may influence IMCL content.

A secondary purpose was to determine whether dietary advice on consuming low fat foods will improve metabolic profiles in adults with abdominal obesity.

A third purpose was to determine whether there is a relationship between the glycemic index and metabolic profiles after following a low fat dietary advice phase for 4 to 6 weeks.

### **3.2 Effects of a eucaloric low glycemic index diet on insulin sensitivity and intramyocellular lipid content in adults with abdominal obesity**

#### **Hypothesis:**

A low glycemic index diet will reduce intramyocellular lipid stores, thereby improving insulin sensitivity in adults with abdominal obesity.

#### **Objective:**

The primary objective was to determine if a 24 week low glycemic index diet will reduce intramyocellular lipid stores, thereby improving insulin sensitivity in individuals with abdominal obesity compared to a high glycemic index diet.

### **3.3 Glycemic index predicts individual glucose responses after self-selected breakfasts in free-living, abdominally obese adults**

#### **Hypothesis:**

The glycemic index is a significant determinant of individual glycemic responses elicited by self-selected breakfast meals in free-living, abdominally obese adults.

#### **Objective:**

The primary objective was to determine if the glycemic index is a significant determinant of individual glycemic responses elicited by self-selected breakfast meals in free-living abdominally obese adults.

**CHAPTER 4**

**EFFECTS OF AN AD-LIBITUM LOW FAT DIET ON METABOLIC PROFILES IN**

**ADULTS WITH ABDOMINAL OBESITY**

## **4. Effects of an ad-libitum low fat diet on metabolic profiles in adults with abdominal obesity**

### **4.1 Introductory Statement**

In both animal and human studies, high fat diets have been shown to increase IMCL storage. In order to examine the role of the glycemic index and IMCL in the primary study, a weight maintaining, ad-libitum low fat dietary advice run-in phase was designed to eliminate potential effects of variations in fat intake on IMCL content.

## 4.2 Abstract

**Background:** Individuals with abdominal obesity are at higher risk for developing cardiovascular disease, type 2 diabetes, and insulin resistance. High fat diets have been shown to increase intramyocellular lipids (IMCL) causing insulin resistance in these individuals. Low fat and low glycemic index (GI) diets may be beneficial in the management of insulin resistance.

**Objectives:** The primary purpose of this low fat, dietary advice study was to eliminate potential effects of variations in dietary fat intake which may influence IMCL content and insulin sensitivity in a primary study.

**Design:** Ninety-five men and non-pregnant, non-lactating women with abdominal obesity, ages 30 to 70 years (mean $\pm$ SD 53.3 $\pm$ 9.5) completed a 4-to-6 week weight maintaining, low fat dietary advice run-in study. The participants completed a 3-day food diary at the start and end of the study. At 4 to 6 weeks, participants underwent a 75g oral glucose test (OGTT) and soleus-muscle IMCL, visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT) were measured by proton nuclear magnetic resonance spectroscopy ( $^1\text{H}$ -MRS).

**Results:** Participants significantly reduced their serum total-, LDL-, and HDL-cholesterol (all,  $p<0.0001$ ), and fasting plasma glucose significantly increased ( $p<0.05$ ). The GI was positively correlated with the insulinogenic index and fasting insulin (both,  $p<0.05$ ). IMCL was positively correlated with waist circumference ( $p<0.01$ ) and BMI, hip circumference, VAT, and total grams of dietary fat (all,  $p<0.05$ ).

**Conclusions:** This study demonstrated that individuals with abdominal obesity can significantly reduce blood lipids by following a weight maintaining, low fat diet.

### 4.3 Introduction

Obesity is a complex disease characterized by an abnormal deposition of fat in adipose tissue and is mainly the result of a positive energy balance where food intake is greater than the body's ability to utilize the food as energy (1). Individuals who have excessive accumulation of adipose tissue, especially in the abdominal region, are at higher risk for developing cardiovascular disease (2, 9), type 2 diabetes (3, 4, 5, 6, 9) and metabolic syndrome and insulin resistance (7, 8). Dietary interventions for these individuals include lowering the overall fat, saturated fat, and cholesterol in the diet (1). One purpose of this study was to determine whether dietary advice on consuming low fat foods will improve metabolic profiles in adults with abdominal obesity.

It has been suggested that individuals with abdominal obesity cannot store lipids effectively in the subcutaneous adipose tissue which in turn leads to an increase in intracellular storage of lipids in organs other than adipose tissue such as the liver, pancreas and skeletal muscle causing insulin resistance in these tissues (10, 11). In animal (12, 13, 14, 15) and human studies (16, 17, 18), high fat diets have been shown to increase skeletal muscle fat, specifically intramyocellular fat (IMCL) and decrease insulin sensitivity. The primary purpose of designing a low fat dietary advice run-in phase prior to the main study was to eliminate potential effects of variations in dietary fat intake which may influence IMCL content and insulin sensitivity.

Low glycemic index carbohydrate foods have also been extensively researched as a possible dietary intervention in the prevention of diseases. The glycemic index (GI) was developed as a classification of the blood glucose-raising potential of the available carbohydrate in foods (19) and is defined as the incremental area under the blood glucose response curve after consuming a 50 gram available-carbohydrate portion of a test food expressed as a percentage of

the response after consuming 50 grams of oral anhydrous glucose or white bread by the same subject (20, 21). Low glycemic index carbohydrate foods have been shown to improve metabolic profiles related to obesity, diabetes and cardiovascular disease among free-living individuals (22). A third purpose of this study was to determine whether there is a relationship between the glycemic index and metabolic profiles after following a low fat dietary advice phase for 4 to 6 weeks.

#### **4.4 Subjects and Methods**

This study was carried out on an outpatient basis at the Hamilton General Hospital, Centre for Cardiovascular Obesity Research and Management, McMaster University, Faculty of Health Sciences. The study protocol was approved by the Hamilton Health Sciences, McMaster University human research ethics board and carried out in accordance with the Declaration of Helsinki as revised in 2000. All participants were given a participant information sheet and gave informed consent to participate in the study (Appendix 8.1). The trial is publicly registered with ClinicalTrials.gov, number NCT00147264.

##### **4.4.1 Protocol**

This thesis chapter describes the results of a 4-to-6 week weight maintaining low-fat dietary advice run-in period of a randomized 2x2 factorial design clinical trial which determined the effects of a low GI diet and telmisartan on intramyocellular lipids (TRIM trial (Telmistartan-Induced Reduction in Intra-Myocellular Lipids)). An initial screening visit occurred two weeks prior to the study to determine whether the participant met the inclusion criteria. The dietary intervention phase of the TRIM trial is discussed in Chapter 5 of this thesis. The results of the telmisartan trial are not reported in this thesis.



#### 4.4.2 Study Sample

A total of 2433 participants from the general population responded through advertisement in local media and underwent a telephone screening process. Of the 171 participants who were invited for an initial screening visit to assess eligibility, 32 participants did not meet the inclusion criteria and 18 refused to participate. Of the 121 enrolled participants, 95 completed the study resulting in a 21% drop out rate. Seven of the subjects dropped out because they wanted to lose weight, 6 left due to personal reasons, 5 participants had time commitment issues, 5 had adverse events and 3 dropped out for other unknown reasons. Men and non-pregnant, non-lactating women aged 30 to 70 years with abdominal obesity, with or without additional features of the metabolic syndrome, were eligible to participate. Abdominal obesity was defined as a waist circumference of >102 cm for males and >88 cm for females (Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (ATP III)) (23). Inclusion criteria included abdominal obesity, ability to provide written informed consent, between 30 and 70 years of age, and the ability and willingness to complete dietary and activity diaries and questionnaires. Exclusion criteria included diabetes or use of any anti-diabetic drug, uncontrolled hypertension, serum triglycerides >10 mmol/L, active malignancy, chronic inflammatory disorders, endocrine, renal or hepatic dysfunction, use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers in the last 3 months, use of a lipid lowering medication-the dose of which had not been stable for at least 3 months, body mass index of >45, intent to lose weight or use weight loss medications during the study, contraindications to magnetic resonance imaging (MRI) such as claustrophobia or metal prostheses, and any dietary

restrictions that would prevent the participants from following the study protocol during the randomization phase of the study (to be discussed in Chapter 5).

#### **4.4.3 Ad-Libitum Low Fat Diet**

The run-in phase consisted of dietary advice on following a standardized low fat diet as outlined by the American Heart Association (24) consisting of 55% energy from carbohydrate, 30% from fat, less than 7% from saturated fat and 15% from protein. Daily energy requirements were estimated according to the Lipid Research Clinic Requirement formula (25) with an additional 300 kcal per day added on for exercise and daily energy expenditure (Appendix 8.2). Diets were prescribed on an ad libitum basis. The aim of the diet was to be weight maintaining and to eliminate potential effects of variations in fat intake on IMCL content. Participants were given an information sheet (Appendix 8.3) to provide dietary advice on following a low fat diet. The participants completed three MEDFICTS dietary assessment questionnaires to assess dietary fat intake (Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (ATP III)) (26) and completed a 3-day food diary at the start and end of the study to assess fat intake and to provide dietary advice (Appendix 8.5). Micronutrients, macronutrients, and GI of test foods were calculated using the Food Processor SQL Nutrition Analysis & Fitness software package version 9.5 (ESHA Research, Salem, OR, USA) with missing values for GI added using the NutriPro diet analysis program (Glycemic Index Laboratories Inc., University of Toronto). The GI was expressed with the GI of glucose = 100. Participants were instructed to maintain their habitual level of physical activity throughout the study. The Baecke habitual physical activity questionnaire was administered at the start and end of the study to determine activity levels (Appendix 8.6) (27).

#### **4.4.4 Anthropometric Measurements, Blood Pressure and Heart Rate**

Height (cm) was measured at the initial screening visit to the nearest 0.1 cm using a wall mounted stadiometer and body weight was measured at every visit to the nearest 0.1 kg on a digital weigh scale. Body mass index (BMI) was calculated as body weight (kg) divided by height ( $m^2$ ). Waist circumference (WC) was measured to the nearest 0.1 cm using the World Health Organization (WHO) method (mid-point between the palpated inferior border of the last rib and upper border of the iliac crest in a horizontal plane at the end of normal expiration) (28). Hip circumference (HC) was measured to the nearest 0.1 cm at the level of the major trochanter (usually around the largest diameter of the buttocks) (28). Waist-to-hip ratio (WHR) was calculated (waist (cm) divided by hip (cm)) from the measurement of the waist and hip circumference. Body composition analysis (body fat percentage) was assessed by bioelectrical impedance analysis as per manufacturer's instructions (BioScan 916, Maltron International Ltd, Rayleigh, Essex, UK). Waist and hip circumference and body fat percentage were measured at the start and end of the study.

Blood pressure (BP) and heart rate (HR) were measured at every visit in the sitting position using an automatic blood pressure monitor (BpTRU®, VSM MedTech Ltd., Vancouver, BC, Canada) following five minutes of seated rest.

#### **4.4.5 Fasting Plasma Glucose and Lipids**

Blood samples were collected from the antecubital vein following a 12 hour fast at the start of the study. Screening blood tests included fasting glucose, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides. Plasma glucose and lipids were analyzed using standard enzymatic procedures and LDL cholesterol was calculated using the Friedewald formula (32).

#### **4.4.6 Oral Glucose Tolerance Test**

An oral glucose tolerance test (OGTT) was performed in the fasting participant (12 hours) at the end of the study. An indwelling catheter was inserted in the forearm and three blood samples for glucose and insulin were taken five minutes apart (-15, -10, -5 minutes). Participants then ingested (0 minutes) a 75 gram solution of dextrose and venous blood samples were obtained again at 30, 60, and 120 minutes for determination of plasma glucose and insulin. Glucose was measured by a glucose oxidase method and serum insulin was measured with an immunometric assay (Diagnostic Products Corporation, Los Angeles, CA). The glucose and insulin data from the OGTT was used to assess insulin sensitivity (homeostatic model assessment index,  $\text{HOMA} = \text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mmol/L)} \text{ divided by } 22.5$  (29)) and insulin secretion (insulinogenic index (ISI) = ratio of change in insulin to change in glucose from 0 to 30 minutes ( $\text{Delta } I_{30} \text{ divided by } \text{Delta } G_{30}$ ) (30)). The HOMA index has been validated with the gold standard euglycemic hyperinsulinemic clamp technique (31). During the OGTT, fasting blood samples were also collected for total, HDL and LDL cholesterol, free fatty acids, and triglycerides. LDL was calculated using the Friedewald formula (32).

#### **4.4.7 Magnetic Resonance Imaging**

Intramyocellular lipid (IMCL) content of the mid-soleus muscle (predominately oxidative muscle fibres) was assessed by proton magnetic resonance spectroscopy ( $^1\text{H}$ -MRS) at the end of the run-in period. MRS was performed on a 1.5 Tesla whole body MR system (Siemens Symphony AG, Munich, Germany) using a body coil for radiofrequency transmission and surface coil to receive signals. The  $^1\text{H}$ -MRS scans were performed at the Nuclear Medicine Department at the McMaster University Medical Centre. Participants were advised to fast and restrict physical activity for six hours prior to the procedure. Following screening for absence of

MRI contraindications, the participant was placed in the supine position and the leg was positioned and immobilized so that the calf was situated as close to the center of the magnet as possible. The radio-frequency receive coil was fastened nearest to the region of interest to collect the greatest signal. Participants remained in the supine position (feet first) within the MR system. Three-plane spin-echo T1-weighted MR images were performed to guide placement of the volume of interest for spectroscopy. Imaging parameters were chosen for suitable separation of muscle, fascia, IMCL and extramyocellular lipid (EMCL) content (TR 3000 milliseconds (ms), TE 30 ms, 128 averages, 1,024 data points over 1000 Hz spectral width, 1 cc voxel volume, water signal suppressed using chemical selective saturation). Semi-automatic shimming of the magnet with typical line widths of the water signal of 10 Hz was performed to optimize magnetic field homogeneity. Several scout images were taken to determine the ideal position for the voxel location. Volume of interest was centered over the mid-soleus muscle and vascular structures and gross adipose tissue deposits were excluded as much as possible. The IMCL and EMCL peak was integrated at 1.28 ppm and 1.48 ppm respectively. Since creatine content is stable within the same muscle across a population (50), spectral intensities were referenced to the methyl signal of creatine ( $\text{Cr}_3$ ) at 3.05 ppm serving as an internal reference. Spectra were processed and the resonance curves for IMCL, EMCL, and creatine were measured using jMRUI software v2.1 (51). Data is presented as arbitrary units.

After 3-plane localizer image acquisition, breath-hold axial T1-weighted image at the level of mid-L4 (TR 400 ms, TE 13 ms) was acquired for the volume of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). VAT and SAT was calculated using SliceOmatic 4.2 medical imaging software (SliceOmatic v.4.2, Tomovision, Montreal). VAT was defined as adipose tissue within the inside edge of the abdominal wall and SAT was defined as adipose

tissue on the outside edge of the abdominal wall. The intra-and-inter-observer coefficients of variation for this method are 0.53% and 0.44% for SAT and 1.46% and 2.42% for VAT respectively.

#### **4.4.8 Statistical Analysis**

Statistical analyses were performed using SPSS 10.1 for Windows (SPSS Inc., Chicago, IL, USA). Pearson product moment correlation coefficient and Student's t-tests were used to determine correlations and differences of means between IMCL, GI, macronutrient composition of the diet, anthropometric data, blood glucose, blood insulin and blood lipid parameters. Statistical significance was set at  $p < 0.05$ . All data are presented as means  $\pm$ SD unless otherwise indicated.

### **4.5 Results**

#### **4.5.1 Subject Characteristics**

Entering the 4 to 6 week study, the majority of the study population were female (73%) and Caucasian (89%), with a mean age of 52 ( $\pm 10$ ) and the majority of the women were menopausal (92%). According to the NCEP-ATP III definition for metabolic syndrome (1), all participants had abdominal obesity (100%) (Waist Circumference (WC)  $> 102$  cm (males),  $> 88$  cm (females), 56% elevated triglycerides ( $> 1.7$  mmol/L), 27% high blood pressure ( $\geq 130/85$ ), 23% low HDL cholesterol ( $< 1.0$  mmol/L (males),  $< 1.3$  mmol/L (females)) and 9% impaired fasting glucose ( $\geq 6.1$  mmol/L). The clinical characteristics of the study population had changed very little by the end of the study (within 3%), however due to drop out rate, 6% more participants had impaired fasting glucose, 10% lower HDL cholesterol, and 23% fewer women were menopausal (Table 4.1).

**Table 4.1.** Clinical Characteristics of the Study Population at Screening, Start and End of Study

	<b>Screening</b>	<b>Start</b>	<b>End</b>
<b>Variable</b>	<b>N = 171</b>	<b>N = 121</b>	<b>N = 95</b>
Age	52 [ $\pm$ 10]	52 [ $\pm$ 10]	53 [ $\pm$ 9]
Male	47 (27)	33 (27)	28 (29)
Female	124 (73)	88 (73)	67 (71)
Menopause	115 (93)	81 (92)	62 (69)
Caucasian	152 (89)	108 (89)	86 (91)
Smoker	60 (35)	41 (34)	34 (36)
Calcium Channel Blocker medication	9 (6)	7 (6)	8 (9)
Beta Blocker medication	12 (7)	8 (7)	5 (6)
Anti-hypertensive medications (excluding ARB)	31 (18)	20 (17)	15 (16)
Diuretics	10 (6)	6 (5)	5 (5)
Aspirin	17(10)	12 (10)	8 (8)
Lipid Lowering Drugs	27 (16)	19 (16)	16 (17)
Abdominal Obesity	171 (100)	121 (100)	95 (100)
Triglycerides $\geq$ 1.7 mmol/L	85 (50)	68 (56)	51 (54)
Blood Pressure $\geq$ 130/85 mmHg	53 (31)	33 (27)	23 (24)
Fasting Glucose 6.1- 6.9 mmol/L	16 (9)	11 (9)	14 (15)
HDL-C < 1.0 mmol/L (M), < 1.3 mmol/L (F)	40 (23)	28 (23)	31 (33)

Numbers in round ( ) brackets represent percentage of the study population. Numbers in square brackets [ ] represent  $\pm$  SD of the mean. M indicates males, F indicates females. ARB designates angiotensin receptor blockers. C stands for cholesterol.

#### 4.5.2 Anthropometric Measurements, Blood Pressure and Heart Rate

At the start-and-end of the study, the participant's mean BMI was  $34.7 \pm 6.1$  and  $34.4 \pm 5.7$  respectively, demonstrating that participants were classified as obese ( $\text{BMI} \geq 30$ ) during the study. BMI, WC, HC, WHR, % body fat, systolic BP, diastolic BP and HR did not significantly change ( $p > 0.05$ ) (Table 4.2).

**Table 4.2.** Anthropometric Characteristics, Blood Pressure and Heart Rate at Baseline and End of Study

	Start	End	
Variable	N = 121	N = 95	p value
BMI ( $\text{kg/m}^2$ )	$34.7 \pm 6.1$	$34.4 \pm 5.7$	0.34
Waist Circumference (cm)	$111.0 \pm 14.5$	$110.5 \pm 12.6$	0.69
Hip Circumference (cm)	$121.4 \pm 12.4$	$121.1 \pm 11.6$	0.43
Waist to Hip Ratio	$0.92 \pm 0.09$	$0.91 \pm 0.08$	0.44
% body fat	$42.2 \pm 9.2$	$41.4 \pm 9.9$	0.10
Systolic Blood Pressure (mmHg)	$122.4 \pm 14.0$	$121.1 \pm 12.2$	0.11
Diastolic Blood Pressure (mmHg)	$78.5 \pm 7.9$	$77.7 \pm 7.7$	0.32
Heart Rate (beats/minute)	$75.4 \pm 12.8$	$72.9 \pm 11.2$	0.07

Data are means  $\pm$ SD

#### 4.5.3 Fasting Plasma Glucose and Lipids

After following dietary advice for a low fat, weight maintaining diet, fasting total serum cholesterol, LDL cholesterol, and HDL cholesterol all significantly decreased ( $p < 0.0001$ ), while



fasting plasma glucose significantly increased ( $p<0.05$ ) (Table 4.3). There was a trend towards a decrease in fasting triglycerides but it was not significant ( $p>0.05$ ) (Table 4.3).

**Table 4.3.** Fasting Plasma Glucose and Lipids and Significance of Changes over the Dietary Period

	Start	End	%	
Variable	N = 121	N = 95	Change	p-value
Total Cholesterol (mmol/L)	5.6 $\pm$ 1.2	5.1 $\pm$ 1.0	-8.9	0.000 **
LDL Cholesterol (mmol/L)	3.3 $\pm$ 1.0	3.0 $\pm$ 0.9	-9.1	0.000 **
HDL Cholesterol (mmol/L)	1.4 $\pm$ 0.4	1.3 $\pm$ 0.3	-7.1	0.000 **
Triglycerides (mmol/L)	2.1 $\pm$ 1.3	2.0 $\pm$ 1.3	-4.8	0.186
Fasting Plasma Glucose (mmol/L)	5.4 $\pm$ 0.6	5.5 $\pm$ 0.8	+1.9	0.036 *

Data are means  $\pm$ SD, \* $p<0.05$ , \*\*  $p<0.0001$

#### 4.5.4 Dietary Intake and Physical Activity

There was a significant decrease in the percentage of energy (kilocalories (kcal)) from total fat ( $p<0.0001$ ) and saturated fat ( $p<0.01$ ) and the MEDFICTS dietary assessment questionnaire of fat intake ( $p<0.00001$ ) (Table 4.4). The percentage of energy (kcal) from carbohydrate and protein, as well as grams of fibre intake significantly increased ( $p<0.01$ ) (Table 4.4). No significant differences were seen in energy intake (total kilocalories), GI, alcohol, or physical activity level ( $p>0.05$ ) (Table 4.4).

**Table 4.4.** Dietary Intake and Physical Activity at the Start and End of the Study

Variable	Start (N = 121)	End (N = 95)	p value
Energy intake (kcal)	2077.4 $\pm$ 625.1	2020.5 $\pm$ 584.8	0.465
Total Fat (g)	79.1 $\pm$ 32.8	70.7 $\pm$ 32.8	0.025 *
Total Fat (% kcal)	33.0 $\pm$ 7.1	29.6 $\pm$ 7.5	0.0000 ****
Saturated Fat (g)	27.2 $\pm$ 22.9	21.5 $\pm$ 9.7	0.017 *
Saturated Fat (% kcal)	10.7 $\pm$ 3.0	9.5 $\pm$ 3.4	0.005**
Total Carbohydrate (g)	253.0 $\pm$ 80.6	260.1 $\pm$ 88.5	0.305
Total Carbohydrate (% kcal)	48.4 $\pm$ 8.4	51.0 $\pm$ 8.5	0.003**
Fibre (g)	20.7 $\pm$ 9.8	23.8 $\pm$ 11.3	0.008 **
Protein (g)	88.7 $\pm$ 30.5	91.2 $\pm$ 29.8	0.453
Protein (%)	17.0 $\pm$ 3.4	18.0 $\pm$ 3.4	0.010 **
Alcohol (%)	3.6 $\pm$ 18.7	1.2 $\pm$ 3.2	0.020*
Glycemic Index (%)	59.7 $\pm$ 4.2	59.8 $\pm$ 3.7	0.931
MEDFICTS score	64.4 $\pm$ 25.8	46 $\pm$ 18.6	0.0000*****
Baecke Activity	2.4 $\pm$ 0.6	2.5 $\pm$ 0.5	0.417

Data are means  $\pm$ SD, \*p<0.05, \*\*p<0.01, \*\*\*p<0.0001, \*\*\*\*p<0.00001

% kcal;percentage of total energy, Baecke Activity;Baecke activity questionnaire

#### **4.5.5 Oral Glucose Tolerance Test**

##### **4.5.5.1 Insulin and Glucose**

Upon completion of the study, there was a significant positive correlation with the insulinogenic index (ISI) and percentage body fat and the MEDFICTS dietary fat questionnaire (both,  $p < 0.05$ ), and a negative correlation between ISI and activity level (both,  $p < 0.05$ ). HOMA was positively correlated with BMI, WC, and visceral adipose tissue (VAT) (all,  $p < 0.0001$ ), HC and percentage of body fat (both,  $p < 0.001$ ), percentage of energy from dietary fat and grams of saturated fat (both,  $p < 0.05$ ), and negatively correlated with HDL cholesterol ( $p < 0.01$ ).

Fasting insulin was positively correlated with BMI, VAT, WC and HC (all,  $p < 0.001$ ), percentage of body fat ( $p < 0.01$ ), and grams of saturated fat, percentage of energy from dietary fat, and GI (all,  $p < 0.05$ ), and negatively correlated with HDL cholesterol and activity level (both,  $p < 0.05$ ). Thirty minute insulin was positively correlated with the MEDFICTS dietary fat questionnaire ( $p < 0.01$ ), and BMI, VAT, percentage of body fat, and percentage of energy from dietary fat and saturated fat (all,  $p < 0.05$ ), and negatively correlated with activity level, dietary fibre (g) and percentage of energy from carbohydrate (all,  $p < 0.05$ ).

Fasting glucose was positively correlated with VAT ( $p < 0.05$ ). Thirty minute glucose was positively correlated with VAT ( $p < 0.05$ ) and percentage of energy from saturated fat and total fat (both,  $p < 0.01$ ), and negatively correlated with dietary fibre (g) and percentage of energy from carbohydrate (both,  $p < 0.05$ ). Sixty minute glucose was positively correlated with VAT and WC (both,  $p < 0.01$ ) and negatively correlated with HDL cholesterol ( $p < 0.001$ ). Glucose at 120 minutes was positively correlated with free fatty acids (FFA) ( $p < 0.05$ ) and negatively correlated with carbohydrate (g) ( $p < 0.05$ ). Refer to Table 4.5 for insulin and glucose correlations.

**Table 4.5. Correlation Grid for Insulin and Glucose**

	<b>GI</b>	<b>MED-</b>	<b>Total</b>	<b>Total</b>	<b>Sat</b>	<b>Sat</b>	<b>Fibre</b>	<b>CHO</b>	<b>CHO</b>
	<b>%</b>	<b>FICTS</b>	<b>Fat %</b>	<b>Fat (g)</b>	<b>Fat %</b>	<b>Fat (g)</b>	<b>(g)</b>	<b>%</b>	<b>(g)</b>
<b>ISI</b>	0.22	<b>0.26*</b>	0.10	0.13	0.09	0.17	0.02	-0.07	0.10
<b>HOMA</b>	0.19	0.14	<b>0.23*</b>	0.21	0.19	<b>0.22*</b>	0.02	-0.07	0.10
<b>FastIns</b>	<b>0.21*</b>	0.16	<b>0.22*</b>	0.21	0.19	<b>0.23*</b>	-0.15	-0.14	-0.01
<b>30mIns</b>	0.18	<b>0.31**</b>	<b>0.25*</b>	0.13	<b>0.25*</b>	0.22	<b>-0.27*</b>	<b>-0.23*</b>	-0.12
<b>30mPG</b>	0.05	0.16	<b>0.32**</b>	0.16	<b>0.31**</b>	0.19	<b>-0.25*</b>	-0.25	-0.15
<b>120mPG</b>	-0.07	-0.08	-0.02	-0.15	0.03	-0.15	-0.05	0.04	<b>-0.21*</b>
	<b>IMCL</b>	<b>VAT</b>	<b>Baecke</b>	<b>%BF</b>	<b>BMI</b>	<b>WC</b>	<b>HC</b>	<b>HDL</b>	<b>FFA</b>
<b>ISI</b>	-0.02	0.02	<b>-0.23*</b>	<b>0.23*</b>	0.16	0.06	0.15	0.01	-0.18
<b>HOMA</b>	0.12	<b>0.53<sup>\$</sup></b>	-0.18	<b>0.38<sup>#</sup></b>	<b>0.51<sup>\$</sup></b>	<b>0.42<sup>\$</sup></b>	<b>0.40<sup>#</sup></b>	<b>-0.30**</b>	-0.17
<b>FastIns</b>	0.10	<b>0.56<sup>\$</sup></b>	<b>-0.21*</b>	<b>0.35**</b>	<b>0.52<sup>\$</sup></b>	<b>0.43<sup>\$</sup></b>	<b>0.40<sup>\$</sup></b>	<b>-0.29*</b>	-0.18
<b>30mIns</b>	-0.01	<b>0.30*</b>	<b>-0.25*</b>	<b>0.26*</b>	<b>0.28*</b>	0.21	0.19	-0.05	-0.12
<b>FPG</b>	0.11	<b>0.26*</b>	0.06	0.13	0.14	0.19	0.08	-0.16	-0.14
<b>30mPG</b>	0.09	<b>0.23*</b>	0.09	0.02	0.06	0.19	0.00	-0.17	-0.02
<b>60mPG</b>	0.15	<b>0.33**</b>	0.05	0.05	0.08	<b>0.27**</b>	0.08	<b>-0.35<sup>#</sup></b>	0.17
<b>120mPG</b>	-0.01	0.16	-0.01	0.21	0.11	0.08	0.08	-0.14	<b>0.26*</b>

The data in the table represent correlation r values. \*p<0.05, \*\*p<0.01, #p<0.001, \$p<0.0001, GI;glycemic index, %;percentage of total energy, sat fat;saturated fat, CHO;carbohydrate, IMCL: intramyocellular lipids (arbitrary units (AU)), VAT;visceral adipose tissue (AU), Baecke; Baecke activity questionnaire, %BF;percentage body fat, BMI;body mass index, WC;waist circumference (cm), HC;hip circumference (cm), HDL;high density lipoprotein cholesterol (mmol/L), FFA;free fatty acids (μmol/L), ISI;insulinogenic index, HOMA;homeostasis model assessment, FastIns;fasting insulin, 30mIns;30 minute insulin, FPG;fasting plasma glucose, 30mPG;30 minute plasma glucose, 60mPG;60 minute plasma glucose, 120mPG;120 minute plasma glucose: plasma glucose was measured in mmol/L, plasma insulin was measured in pmol/L): insulin, glucose, FFA, HDL cholesterol were obtained during the oral glucose tolerance test

#### **4.5.5.2 Blood lipids**

At the end of the run-in period, fasting total cholesterol was negatively correlated with dietary carbohydrate ( $p<0.05$ ). LDL cholesterol was positively correlated with percentage of energy from total fat and negatively correlated with percentage of energy from carbohydrate (both  $p<0.05$ ). Triglycerides were positively correlated with VAT ( $p<0.01$ ). Free fatty acids were positively correlated with percentage of body fat ( $p<0.01$ ) and 120 minute plasma glucose ( $p<0.05$ ) and negatively correlated with WHR, dietary carbohydrate (g) and protein (g) (all,  $p<0.05$ ). HDL cholesterol was negatively correlated with WHR ( $p<0.0001$ ), WC ( $p<0.001$ ), fasting insulin, 60 minute plasma glucose, VAT, HOMA, and dietary carbohydrate and protein (g) (all,  $p<0.01$ ), and fibre ( $p<0.05$ ). Refer to Table 4.6 for blood lipid correlations.

#### **4.5.6 Intramyocellular Lipids (IMCL), Subcutaneous Adipose Tissue (SAT), Visceral Adipose Tissue (VAT)**

At the end of the run-in period, IMCL was positively correlated with VAT ( $r=0.27$ ,  $p<0.05$ ). IMCL was also positively correlated with WC ( $p<0.01$ ), BMI, and HC (both,  $p<0.05$ ), and grams of dietary carbohydrate, fat and protein (all,  $p<0.05$ ) (Table 4.7). IMCL was not significantly correlated with HOMA, ISI, or any insulin, glucose or lipid measurements obtained during the OGTT ( $p>0.05$ ) (Table 4.5, 4.6). Subcutaneous adipose tissue (SAT) was positively correlated with BMI, WC and HC (all,  $p<0.0001$ ) and percentage of body fat ( $p<0.001$ ) (Table 4.7). VAT was positively correlated with BMI, WHR, WC, fasting plasma insulin and HOMA (all,  $p<0.0001$ ), HC ( $p<0.001$ ), triglycerides and 60 minute plasma glucose (both,  $p<0.01$ ), IMCL, 30 minute plasma insulin, MEDFICTS dietary fat questionnaire, fasting and 30 minute plasma glucose, and grams of dietary protein and fat (all,  $p<0.05$ ) (Tables 4.5, 4.6, 4.7). VAT

was negatively correlated with HDL cholesterol ( $p<0.01$ ) and activity level ( $p<0.05$ ) (Tables 4.6, 4.7). The GI was positively correlated with VAT however it was not significant ( $p=0.06$ ).

**Table 4.6. Correlation Grid for Serum Lipids**

	<b>CHO %</b>	<b>CHO (g)</b>	<b>Total Fat %</b>	<b>Protein (g)</b>	<b>Fibre (g)</b>	<b>IMCL</b>		
<b>Total-C</b>	<b>-0.25*</b>	<b>-0.21*</b>	0.20	-0.01	-0.15	-0.17		
<b>LDL-C</b>	<b>-0.26*</b>	-0.14	<b>0.23*</b>	0.07	-0.10	-0.11		
<b>HDL-C</b>	-0.09	<b>-0.29**</b>	0.23	<b>-0.32**</b>	<b>-0.26*</b>	-0.20		
<b>FFA</b>	0.04	<b>-0.23*</b>	-0.03	<b>-0.22*</b>	-0.18	-0.10		
<b>TG</b>	0.01	-0.00	-0.04	0.13	0.07	-0.00		
	<b>VAT</b>	<b>WC</b>	<b>WHR</b>	<b>%BF</b>	<b>FastIns</b>	<b>HOMA</b>	<b>60mPG</b>	<b>120mPG</b>
<b>HDL-C</b>	<b>-0.32**</b>	<b>-0.33<sup>#</sup></b>	<b>-0.44<sup>\$</sup></b>	0.07	<b>-0.29**</b>	<b>-0.30**</b>	<b>-0.35**</b>	-0.14
<b>FFA</b>	-0.11	-0.02	<b>-0.23*</b>	<b>0.30**</b>	-0.18	-0.17	0.17	<b>0.26*</b>
<b>TG</b>	<b>0.31**</b>	0.04	0.11	0.13	0.13	0.14	0.15	0.11

The data in the table represent correlation  $r$  values. \* $p<0.05$ , \*\* $p<0.01$ , <sup>#</sup> $p<0.001$ , <sup>\$</sup> $p<0.0001$ , %;percentage of total energy, CHO;carbohydrate, IMCL;intramyocellular lipids (arbitrary units (AU)), VAT;visceral adipose tissue (AU), %BF;percentage body fat, WC;waist circumference (cm), Total-C;total cholesterol (mmol/L), HDL;high density lipoprotein cholesterol (mmol/L), LDL-C;low density lipoprotein cholesterol (mmol/L), FFA;free fatty acids ( $\mu\text{mol/L}$ ), TG;triglycerides (mmol/L), HOMA;homeostasis model assessment index, FastIns;fasting plasma insulin (pmol/L), 60mPG;60 minute plasma glucose (mmol/L), 120mPG;120 minute plasma glucose (mmol/L): FFA, insulin, glucose and cholesterol were obtained during the oral glucose tolerance test

**Table 4.7. Correlation Grid for IMCL, SAT, VAT**

	<b>BMI</b>	<b>WC</b>	<b>HC</b>	<b>%BF</b>	<b>WHR</b>	<b>Baecke</b>
<b>IMCL</b>	<b>0.22*</b>	<b>0.31**</b>	<b>0.23*</b>	0.04	0.21	-0.04
<b>SAT</b>	<b>0.68<sup>\$</sup></b>	<b>0.54<sup>\$</sup></b>	<b>0.81<sup>\$</sup></b>	<b>0.41<sup>#</sup></b>	-0.12	-0.21
<b>VAT</b>	<b>0.48<sup>\$</sup></b>	<b>0.65<sup>\$</sup></b>	<b>0.37<sup>#</sup></b>	0.13	<b>0.50<sup>\$</sup></b>	<b>-0.25*</b>

	<b>MEDFICTS</b>	<b>Protein (g)</b>	<b>Total Fat (g)</b>	<b>CHO (g)</b>
<b>IMCL</b>	0.05	<b>0.24*</b>	<b>0.22*</b>	<b>0.28*</b>
<b>VAT</b>	<b>0.23*</b>	<b>0.25*</b>	<b>0.25*</b>	0.05

The data in the table represent correlation r values. \*p<0.05, \*\*p<0.01, #p<0.001, \$p<0.0001, IMCL;intramyocellular lipid (arbitrary units (AU)), SAT;subcutaneous adipose tissue (AU), VAT;visceral adipose tissue (AU), BMI;body mass index, WC;waist circumference (cm), HC;hip circumference (cm), %BF;percentage body fat, WHR;waist to hip ratio, Baecke;Baecke activity questionnaire, CHO;carbohydrate.

#### **4.6 Discussion and Conclusions**

This study demonstrated that individuals with abdominal obesity can significantly reduce blood lipids by following dietary advice on consuming an ad-libitum low fat diet without any strict dietary protocol to follow and without losing weight or increasing physical activity. At the start-and-end of the study, the participant's mean BMI was 34.7±6.1 and 34.4±5.7 respectively, demonstrating that participants were classified as obese (BMI≥30) during the study. Total energy (kcal) intake, activity level, BMI, WC, hip circumference, WHR, and percentage body fat did not significantly change (p>0.05) indicating that participants followed a weight maintaining diet. There was a significant decrease in the percentage of total dietary fat (p<0.0001) and saturated

fat ( $p<0.01$ ) and the MEDFICTS score (dietary assessment questionnaire of fat intake) ( $p<0.00001$ ) demonstrating that the participant's lowered their fat intake during the study.

The recommended NCEP-ATP III dietary interventions for obese individuals, specifically to prevent coronary heart disease, include weight loss, physical activity and lowering the overall fat, saturated fat, and cholesterol in the diet, with the primary goal of lowering LDL cholesterol (1). This study provided dietary advice for following the NCEP-ATP III dietary recommendations for lowering the overall fat and saturated fat. Participants reduced the saturated fat and total fat in their diet while increasing the carbohydrate and protein in the diet. The significant reductions ( $p<0.0001$ ) in total cholesterol (-8.9% change) and LDL cholesterol (-9.1% change) are consistent with other studies showing a reduction in serum lipids when following a low fat diet (33, 37). A meta-analysis by Clarke et al (33) reported that replacing saturated fat with complex carbohydrate can decrease total serum cholesterol by 0.52 ( $SE\pm 0.03$ ) mmol/L and LDL cholesterol by 0.36 ( $SE\pm 0.05$ ) mmol/L. Other studies have shown an increase in triglycerides and a decrease in HDL cholesterol when replacing saturated fat with carbohydrate (34, 35), however, when fibre is increased along with carbohydrate intake, the negative effects are reduced (35, 36). In this study, there were no significant changes in triglycerides after following an ad-libitum low fat diet, however, HDL cholesterol decreased significantly ( $p<0.0001$ ) even though fibre intake increased throughout the study. An explanation for the reduction in HDL cholesterol is that all serum lipids will be reduced when restricting fat intake in the diet. Reductions in HDL cholesterol have been previously reported when following a low fat diet (37) which prompted the NCEP-ATP III to include physical activity and weight reduction along with following a low fat diet to reduce cardiovascular disease risk (1). The focus of this study was to eliminate potential effects of variations in dietary fat



intake which may influence IMCL content and not to lower serum lipids, therefore physical activity and weight loss were not part of the protocol in this study. This study did demonstrate that a weight maintaining, low fat, ad-libitum diet for 4 to 6 weeks can significantly reduce serum lipids in obese individuals. Thus, a weight-maintaining, low fat diet may reduce an individuals' risk for developing cardiovascular disease if followed for a longer duration.

In animal (12, 13, 14, 15) and human studies (16, 17, 18), high fat diets have been shown to increase IMCL and decrease insulin sensitivity. In this study, IMCL was positively correlated with total dietary fat ( $p < 0.05$ ), which is consistent with studies showing a relationship with dietary fat and IMCL however there were no significant correlations between IMCL and insulin sensitivity. A possible explanation for not finding correlations with IMCL and insulin sensitivity in this study is participants were given dietary advice regarding food choices for following a low fat diet whereas other studies provided controlled high fat feedings and diets. Further, studies demonstrating a relationship between insulin resistance and IMCL used the euglycemic hyperinsulinemic clamp method (16, 17, 45). The current study did not find a correlation between insulin resistance and IMCL possibly due to using the HOMA index to estimate insulin sensitivity. The results of the current study are in agreement with previous studies using HOMA that did not find a relationship between IMCL and insulin sensitivity (46, 47). Although HOMA has been validated with the euglycemic hyperinsulinemic clamp technique (31), it is more a measure of hepatic insulin resistance rather than an indicator of muscle insulin sensitivity. It may be possible that the relationship between insulin resistance and IMCL may not be found when using HOMA and a more accurate technique may have identified this relationship in the current study. Further, no relationship was found between IMCL and two hour plasma glucose (120mPG) after OGTT which is an indicator of muscle insulin sensitivity. The lack of

relationship between IMCL and two hour plasma glucose may be due to the fact that the study population was abdominally obese but otherwise healthy individuals with fasting and 120 minute glucose and insulin within recommended ranges. The current results are consistent with other studies that found no correlation between IMCL and insulin sensitivity in healthy, overweight individuals (46, 48, 49). Further, a limitation of this run-in phase was that IMCL content was measured only at the end of the study, therefore conclusions cannot be made on whether changes occurred in IMCL and if there was any effect on insulin sensitivity after reducing fat in the diet. On the other hand, IMCL was positively correlated with VAT ( $p<0.05$ ) and VAT was positively correlated with the OGTT fasting insulin ( $p<0.00001$ ) and glucose ( $p<0.05$ ), 30 minute insulin and glucose ( $p<0.05$ ), 60 minute glucose ( $p<0.01$ ) and HOMA ( $p<0.0001$ ). Findings are consistent with a study by Koska et al (38) who found a positive correlation with fasting plasma insulin and VAT ( $p<0.01$ ) but no correlation with IMCL and insulin action on glucose uptake and production in obese individuals. The results of this study showed that IMCL and VAT were correlated with fat intake however VAT may independently have an effect on insulin sensitivity.

A third purpose of this study was to determine whether there was a relationship between the glycemic index and metabolic profiles after following a low fat dietary advice phase for 4 to 6 weeks. Although participants were not given dietary advice on following a low GI diet, there was a significant positive relationship between glycemic index (GI) and fasting insulin ( $p<0.05$ ). Wolever et al (41, 42) have previously shown that individual foods with different GI's as well as mixed meals varying in GI affect postprandial plasma insulin and glucose differently. Although this study did not find a correlation between GI and plasma glucose, research has shown a relationship between mean plasma insulin and glucose response areas under the curve with foods varying in GI (41). Findings from this study are consistent with previous research demonstrating

that insulin is positively correlated with the GI, indicating that lower GI foods can be less demanding on the pancreas for insulin production (43, 44). Since abdominally obese individuals are susceptible to developing insulin resistance, following a diet with lower GI foods may have metabolic benefits such as improving insulin sensitivity and should be evaluated further.

In conclusion, this study has shown that a weight maintaining, low-fat, ad-libitum diet can reduce total serum cholesterol and LDL serum cholesterol in abdominally obese individuals, a population at risk for coronary heart disease, diabetes, and metabolic syndrome. These findings may help to explain the importance of a low fat diet, especially for those individuals who do not wish to reduce body weight or increase activity level. The participants in this study obtained dietary advice on following a low fat diet for a short period of time. To confirm the present findings, research is needed to determine whether individuals can follow dietary advice on following a low fat diet for a longer duration. Further, participants in this study did not receive dietary advice on following a low GI diet. Future research is needed to determine the long-term effects of a non-prescribed, ad-libitum, low fat, low GI diet on metabolic profiles in free-living individuals with abdominal obesity.

## 4.7 References

1. World Health Organization. Obesity: preventing and managing the global epidemic: report of a WHO consultation. WHO Technical Report Series No. 894. 2000. Geneva, Switzerland: World Health Organization.
2. Wilson PW, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Archives of Internal Medicine* 2002;162:1867-1872.
3. Bray GA, Jablonski KA, Fujimoto WY, Barrett-Connor E, Haffner S, Hanson RL, Hill JO, Hubbard V, Kriska A, Stamm E, Pi-Sunyer FX. Relation of central adiposity and body mass index to the development of diabetes in the Diabetes Prevention Program. *American Journal of Clinical Nutrition* 2008;87:1212-1218.
4. Carey VJ, Walters EE, Colditz GA, Solomon CG, Willett WC, Rosner BA, Speizer FE, Manson JE. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. *American Journal of Epidemiology* 1997;145:614-619.
5. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 1994;17:961-969.
6. Wang Y, Rimm EB, Stampfer MJ, Willen WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. *American Journal of Clinical Nutrition* 2005;81:555-563.
7. Grundy, SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith Jr SC, Spertus JA, Costa F. Diagnosis and management of

- the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Executive summary. *Cardiology Reviews* 2005;13:322-327.
8. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005;365:1415-1428.
  9. Balkau B, Deanfield JE, Despres JP, Bassand JP, Fox, KAA, Smith SC Barter P, Tan C, Van Gaal L, Wittchen HU, Massien C, Haffner SM. International day for the evaluation of abdominal obesity (IDEA): A study of waist circumference, cardiovascular disease, and diabetes mellitus in 168 000 primary care patients in 63 countries. *Circulation* 2007;116:1942-1951.
  10. McGarry JD, Dobbins RL. Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 1999;42:128-138.
  11. Shulman GI. Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy. *Physiology* 2004;19:183-190.
  12. Storlien LH, James DE, Burleigh KM, Chisholm DJ, Kraegen EW. Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. *American Journal of Physiology, Endocrinology & Metabolism* 1986;14:E576-E583.
  13. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes* 1991;40:280-289.

14. Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH. Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes* 1991;40:1397-1407.
15. Oakes ND, Cooney GJ, Camilleri S, Chisholm DJ, Kraegen EW. Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. *Diabetes* 1997;46:1768-1774.
16. Boden G, Lebed B, Schatz M, Homko C, Lemieux S. Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes* 2001;50:1612-1617.
17. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, Loviscach M, Stumvoll M, Claussen CD, Schick F, Haring HU, Jacob S. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 2001;50:2579-2584.
18. Schrauwen-Hinderling VB, Kooi ME, Hesselink MKC, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie G, Saris WHM, Nicolay K, Schrauwen P. Intramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet. *Obesity Research* 2005;13:2088-2094.
19. Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 1981;34:362-366.
20. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. *American Journal of Clinical Nutrition* 1991;54:846-854.

21. Wolever TM. Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values. *British Journal of Nutrition* 2004;91:295-300.
22. Ludwig DS. The glycemic index. Physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *Journal of the American Medical Association* 2002;287:2414-2423.
23. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment on high blood cholesterol in adults (Adult Treatment Panel III). *Journal of the American Medical Association* 2001;285:2486-2497.
24. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St.Jeor S, Suttie J, Tribble DL Bazzarre TL. AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the nutrition committee of the American Heart Association. *Circulation* 2000;102:2284-2299.
25. The lipid research clinics population studies data book. Volume 2. The prevalence study-nutrient intake. Washington, D.C. Government Printing Office, National Institutes of Health. NIH Publication No. 82-2014, September 1982.
26. National Cholesterol Education Program National Heart, Lung, and Blood Institute. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Executive Summary, National Institutes of Health. NIH Publication No. 01-3670, May 2001.

27. Baecke JAH, Burema J, Frijters, JER. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *American Journal of Clinical Nutrition* 1982;36:936-942.
28. World Health Organization. Physical Status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. Technical Report Series No 854, Annex 2. 1995;854:1-452.
29. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
30. Pacini G, Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. *Best Practice Research Clinical Endocrinology and Metabolism* 2003;17:305-322.
31. Saad MF, Anderson RL, Laws A, Watanabe RM, Kades WW, Chen YDI, Sands RE, Pei D, Savage PJ, Bergman RN. The Insulin Resistance Atherosclerosis Study: Comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. *Diabetes* 1994;43:1114-1121.
32. Friedewald WT, Levy RT, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. *Clinical Chemistry* 1972;18:499-502.
33. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. *British Medical Journal* 1997;314:112-117.



34. Mensink RP, Katan MB. Effects of dietary fatty acids on serum lipids and lipoproteins. *Arteriosclerosis and Thrombosis* 1992;12:911-919.
35. Turley ML, Skeaff CM, Mann JI, Cox B. The effect of a low-fat, high-carbohydrate diet on serum high density lipoprotein cholesterol and triglyceride. *European Journal of Clinical Nutrition* 1998;52:728-732.
36. Jenkins DJA, Wolever TMS, Venketeshwer Rao A, Hegele RA, Mitchell SJ, Ransom TPP, Boctor DL, Spadafora PJ, Jenkins AL, Mehling C, Katzman R, Lelle L, Connelly PW, Story JA, Furumoto EJ, Corey P, Wursch P. Effects on blood lipids of very high intakes of fiber in diets low in saturated fat and cholesterol. *New England Journal of Medicine* 1993;329:21-26.
37. Schaefer EJ, Levy RI, Ernst ND, Van Sant FD, Brewer HB. The effects of low cholesterol, high polyunsaturated fat, and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholesterolemic subjects. *American Journal of Clinical Nutrition* 1981;34:1758-1763.
38. Koska J, Stefan N, Permana PA, Weyer C, Sonoda M, Bogardus C, Smith SR, Joannisse DR, Funahashi T, Krakoff J, Bunt JC. Increased fat accumulation in liver may link insulin resistance with subcutaneous abdominal adipocyte enlargement, visceral adiposity, and hypoadiponectinemia in obese individuals. *American Journal of Clinical Nutrition* 2008;87:295-302.
39. Rizkalla SW, Taghrid L, Laromiguire M, Huet D, Boillot J, Rigoir A, Elgrably F, Slama G. Improved plasma glucose control, whole-body glucose utilization, and lipid profile on a low-glycemic index diet in type 2 diabetic men. *Diabetes Care* 2004;27:1866-1872.

40. Jarvi AE, Karlstrom BE, Granfeldt YE, Bjorck IE, Asp NL, Vessby EOH. Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients. *Diabetes Care* 1999;22:10-18.
41. Wolever TMS, Bolognesi C. Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. *Journal of Nutrition* 1996;126:2798-2806.
42. Wolever TMS, Bolognesi C. Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index. *Journal of Nutrition* 1996;126:2807-2812.
43. Brand Miller J, Pang E, Broomhead L. The glycaemic index of foods containing sugars: comparison of foods with naturally occurring versus added sugars. *British Journal of Nutrition* 1995;73:613-623.
44. Bjorck I, Liljeberg H, Ostman E. Low glycaemic-index foods. *British Journal of Nutrition* 2000;83:S149-S155.
45. Krssak M, Falk Petersen K, Dresner A, Dipietro L, Vogel SM, Rothman DL, Roden M, Shulman GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a  $^1\text{H}$  NMR spectroscopy study. *Diabetologia* 1999;42:113-116.
46. St-Onge MP, Newcomer BR, Buchthal S, Aban I, Allison DB, Bosarge A, Gower B. Intramyocellular lipid content is lower with a low-fat diet than with high-fat diets, but that may not be relevant for health. *American Journal of Clinical Nutrition* 2007;86:1316-1322.
47. Stannard SR, Holdaway MA, Sachinwalla T, Cunningham TS. Insulin sensitivity and intramyocellular lipid concentrations in young Maori men. *Diabetes Medicine* 2007;24:1205-1212.

48. Goff LM, Frost GS, Hamilton G, Thomas EL, Dhillo WS, Dornhorst A, Bell JD. Carbohydrate-induced manipulation of insulin sensitivity independently of intramyocellular lipids. *British Journal of Nutrition* 2003;89:365-374.
49. Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, Smith SR, Alfonso A, Ravussin E. Effect of calorie restriction with or without exercise on insulin sensitivity,  $\beta$ -cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care* 2006;29:1337-1344.
50. Boesch C, Slotboom J, Hoppeler H, Kreis R. In vivo determination of intra-myocellular lipids in human muscle by means of localized  $^1\text{H}$ -MR-spectroscopy. *Magnetic Resonance in Medicine* 1997;37:484-493.
51. Naressi A, Couturier C, Castang I, de Beer R, Graveron-Demilly D. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. *Computers in Biology and Medicine* 2001;31:269-286.

**CHAPTER 5**

**EFFECTS OF A EUCALORIC LOW GLYCEMIC INDEX DIET ON INSULIN  
SENSITIVITY AND INTRAMYOCYLLULAR LIPIDS IN ADULTS WITH  
ABDOMINAL OBESITY**

## **5. Effects of a eucaloric low glycemic index diet on insulin sensitivity and intramyocellular lipids in adults with abdominal obesity**

### **5.1 Introductory Statement**

Research has shown that insulin sensitivity is negatively correlated with intramyocellular lipid (IMCL) content. It has been suggested that low glycemic index diets may improve insulin sensitivity by suppressing non-esterified fatty acid release, which in turn may promote glucose uptake and possibly lower IMCL content. This study was conducted to determine whether a long term eucaloric low glycemic index diet can improve insulin sensitivity by reducing IMCL content of skeletal muscle in abdominally obese individuals.

## 5.2 Abstract

**Background:** Abdominal obesity is associated with insulin resistance and increased IMCL content of skeletal muscle. Low-glycemic-index (GI) diets may improve insulin sensitivity in insulin resistant subjects but their effect on IMCL is unknown.

**Objective:** The purpose of this study was to determine the effect a low-GI diet (LGID) on insulin sensitivity and IMCL content in weight-stable abdominally obese adults.

**Design:** Ninety-five men and non-pregnant, non-lactating women aged  $53 \pm 10$  years with abdominal obesity were randomized to a 24-week intervention of either a weight-maintaining low glycemic index diet (LGID, n=48) or control high-GI diet (HGID, n=47).  $^1\text{H}$ -MRS of the soleus muscle and an oral glucose tolerance test were performed at the beginning and end of the study to determine IMCL content and insulin sensitivity.

**Results:** On the LGID there were significant decreases from baseline in diet GI ( $p < 0.0001$ ), intakes of total and saturated fat ( $p < 0.001$ ), and 60 minute postprandial glucose ( $p < 0.05$ ), while intakes of carbohydrate and fibre increased ( $p < 0.0001$ ). On the HGID there were significant increases in diet GI ( $p < 0.001$ ), intakes of carbohydrate ( $p < 0.001$ ), total kcal intake ( $p < 0.01$ ), insulinogenic index ( $p < 0.01$ ) and BMI ( $p < 0.05$ ), and a significant decrease in systolic blood pressure ( $p < 0.05$ ). There were no significant changes in IMCL or other metabolic measures for either group.

**Conclusions:** A LGID reduced the OGTT 60 minute plasma glucose but did not significantly affect IMCL or insulin sensitivity in individuals with abdominal obesity.

### 5.3 Introduction

Obesity is a complex disease characterized by an abnormal deposition of fat in adipose tissue and is mainly the result of a positive energy balance where food intake is greater than the body's ability to utilize the food as energy (1). The excessive accumulation of adipose tissue leads to an undesirable weight gain to the extent that health may be adversely affected (1). Obesity, in particular abdominal obesity, has been shown to be a risk factor for developing cardiovascular disease (2, 12), type 2 diabetes (3, 4, 5, 6, 12) and metabolic syndrome (7, 8). Insulin resistance, a key feature of metabolic syndrome, is higher in individuals with overall adiposity (9) and central abdominal obesity (10, 11). Although the mechanism of insulin resistance is not fully understood, it has been suggested that individuals with abdominal obesity cannot store lipids effectively in the subcutaneous adipose tissue which in turn leads to an increase in storage of lipids in the visceral area and in organs other than adipose tissue such as the liver, pancreas and skeletal muscle causing insulin resistance in these tissues (13, 14). Research has shown that muscle fat is increased in individuals with insulin resistance (15) and that insulin sensitivity is negatively correlated with intramyocellular lipid (IMCL) content as assessed by examination from proton nuclear magnetic resonance spectroscopy ( $^1\text{H}$ -MRS), a non-invasive quantification of IMCL content in human muscle that can identify the relative contributions of IMCL and whole body insulin sensitivity (16).

Evidence supports the role of carbohydrates in influencing insulin sensitivity by improving insulin-stimulated glucose uptake by adipocytes when following a low glycemic index diet (17). The glycemic index (GI) was developed as a classification of the blood glucose-raising potential of the available carbohydrate in foods (43) and is defined as the incremental area under the blood glucose response curve after consuming a 50 gram available-carbohydrate

portion of a test food expressed as a percentage of the response after consuming 50 grams of oral anhydrous glucose by the same subject (44, 45). Low glycemic index (GI) carbohydrates are slowly digested and release glucose gradually into the blood stream and therefore may suppress plasma concentrations of fatty acids (18, 19). The suppression of plasma fatty acids may improve insulin sensitivity by promoting insulin-stimulated glucose uptake by skeletal muscle and possibly reduce IMCL storage (19). A four week intervention study examining the effects of a reduction in dietary GI on IMCL in healthy individuals showed improvements in insulin sensitivity but no changes in IMCL storage levels (20). It still remains unclear whether a low GI diet can improve IMCL stores in muscles of individuals who are at risk for developing insulin resistance or type 2 diabetes.

The primary purpose of this 24 week intervention study was to determine whether a low GI diet would reduce IMCL stores thereby improving insulin sensitivity in individuals with abdominal obesity.

#### **5.4 Subjects and Methods**

This study was carried out on an outpatient basis at the Hamilton General Hospital, Centre for Cardiovascular Obesity Research and Management, McMaster University, Faculty of Health Sciences. The study protocol was approved by the Hamilton Health Sciences, McMaster University human research ethics board and carried out in accordance with the Declaration of Helsinki as revised in 2000. All participants were given a participant information sheet and gave informed consent to participate in the study (Appendix 8.1). The trial is publicly registered with ClinicalTrials.gov, number NCT00147264.



### **5.4.1 Protocol**

This thesis chapter consists of a 24 week low GI diet which is the randomization phase of a parallel, 2x2 factorial design clinical trial that studied the effects of a low GI diet and telmisartan, an angiotensin receptor blocker, on intramyocellular lipids (TRIM trial (Telmisartan-Induced Reduction in Intra-Myocellular Lipids)).

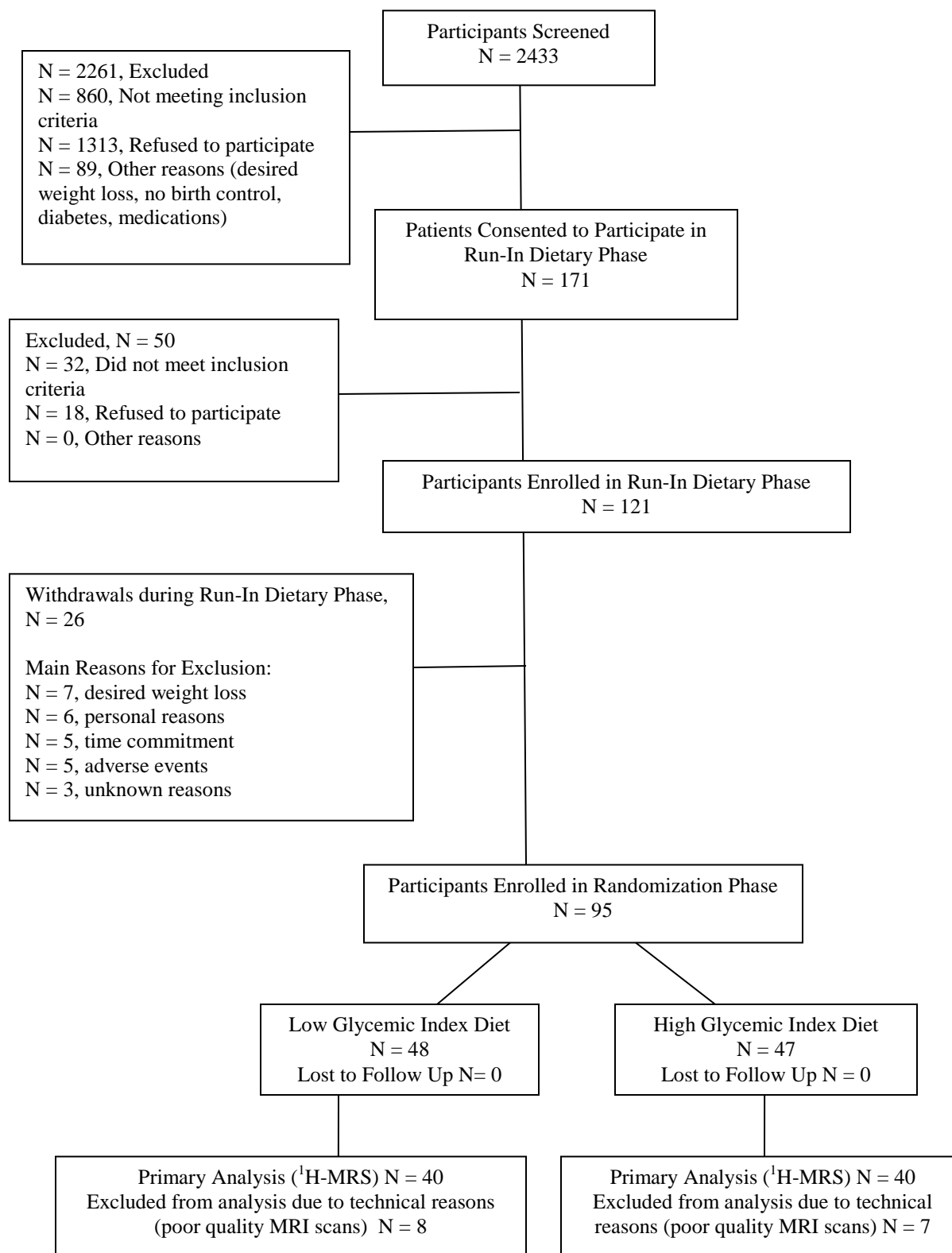
### **5.4.2 Study Sample**

A total of 2433 individuals from the general population responded through advertisement in local media and underwent a telephone screening process. Of the 171 people who were invited for an initial screening visit to assess eligibility, 32 did not meet the inclusion criteria and 18 refused to participate. Of the 121 enrolled participants, 95 completed the run-in phase of the study resulting in a 21% drop out rate. Of the 95 men, and non-pregnant, non-lactating women aged 30 to 70 years with abdominal obesity, with or without additional features of the metabolic syndrome, who were eligible to participate in the randomization study, 48 participants were randomized to the low GI diet (LGID) and 47 participants were randomized to the control, high GI diet (HGID) (Figure 5.1). For the primary outcome (<sup>1</sup>H-MRS), 12 participants in the LGID and 10 participants in the HGID had poor quality MRI scans and were excluded in the data analysis (Figure 5.1). Abdominal obesity was defined as a waist circumference of >102 cm for males and >88 cm for females (NCEP, ATP III) (21). Inclusion criteria included abdominal obesity, ability to provide written informed consent, age between 30 and 70 years, and the ability and willingness to complete dietary and activity diaries and questionnaires. Exclusion criteria included diabetes or use of any anti-diabetic drug, uncontrolled hypertension, serum triglycerides >10 mmol/L, active malignancy, chronic inflammatory disorders, endocrine, renal or hepatic dysfunction, use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers in

the last 3 months, use of a lipid lowering medication-the dose of which had not been stable for at least 3 months, body mass index of  $>45$ , intent to lose weight or use weight loss medications during the study, contraindications to magnetic resonance imaging (MRI) such as claustrophobia or metal prostheses, and any dietary restrictions that would prevent the participants from following the study protocol.

#### **5.4.3. Dietary Intervention**

Participants were randomly assigned to either a low glycemic index diet (LGID, n=48) or a control, high glycemic index diet (HGID, n=47) for 24 weeks by using an automated randomization system. The aim of both diets was to be weight maintaining consisting of 55% of energy from carbohydrate, 30% from fat, 15% from protein and 7% or less from saturated fats as outlined by the American Heart Association (22). Forty percent of the total carbohydrate consisted of either low glycemic index (GI) foods or high GI foods. Participants were provided with low GI and high GI test foods on a monthly basis (Appendix 8.7) and were asked to keep daily dietary records of the number of test foods they consumed each day (Appendix 8.7). The number of test foods to be eaten each day was calculated based on the participant's daily estimated energy requirements according to the Lipid Research Clinic Requirement formula (23) with an additional 300 kilocalories (kcal) per day added on for exercise and daily energy expenditure (Appendix 8.2). One serving of test food contained approximately 15 grams of available carbohydrate. Participants were asked to consume two servings of test foods with the first meal of each day, with the remainder of the test foods to be consumed throughout the day. Weekly compliance of test foods was calculated as the number of test foods consumed divided by the number of test foods required multiplied by 100.



**Figure 5.1. Participant Recruitment Flow Chart**

Participants met seven times during the study for dietary advice, the collection and dispensing of daily food records, analysis of diet compliance, dispensing of test foods, and completing the MEDFICTS dietary assessment questionnaire (NCEP, ATP III) (Appendix 8.4) (21). The 3-day food diaries were analyzed at baseline, and weeks 4, 12, and 24 (Appendix 8.5). Micronutrients, macronutrients, and the GI of foods were calculated using the Food Processor SQL Nutrition Analysis & Fitness software package version 9.5 (ESHA Research, Salem, OR, USA) with missing values for GI added using the NutriPro diet analysis program (Glycemic Index Laboratories Inc., University of Toronto). The GI was expressed with the GI of glucose=100. Participants were instructed to maintain their habitual level of physical activity throughout the study. The Baecke habitual physical activity questionnaire was administered at the start-and-end of the study to determine activity levels (Appendix 8.6) (24).

#### **5.4.4 Anthropometric Measurements, Blood Pressure and Heart Rate**

Height (cm) was measured at the initial screening visit to the nearest 0.1 cm using a wall mounted stadiometer and body weight was measured at every visit to the nearest 0.1 kg on a digital weigh scale. Body mass index (BMI) was calculated as body weight (kg) divided by height ( $m^2$ ). Waist circumference (WC) was measured to the nearest 0.1 cm using the World Health Organization (WHO) method (mid-point between the palpated inferior border of the last rib and upper border of the iliac crest in a horizontal plane at the end of normal expiration) (25). Hip circumference (HC) was measured to the nearest 0.1 cm at the level of the major trochanter (usually around the largest diameter of the buttocks) (25). Waist-to-hip ratio (WHR) was calculated (waist (cm) divided by hip (cm)) from the measurement of the waist and hip circumference. Body composition analysis (percentage of body fat) was assessed by bioelectrical impedance analysis as per manufacturer's instructions (BioScan 916, Maltron International Lt,

Rayleigh, Essex, UK). Waist and hip circumference and body fat percentage were measured at the start, middle and end of the study.

Blood pressure (BP) (mmHg) and heart rate (HR) (beats per minute) were measured at every visit in the sitting position using an automatic blood pressure monitor (BpTRU®, VSM MedTech Ltd., Vancouver, BC, Canada) following five minutes of seated rest.

#### **5.4.5 Oral Glucose Tolerance Test**

An oral glucose tolerance test (OGTT) was performed in the fasting participant (12 hours) at the start, middle and end of the study. An indwelling catheter was inserted in the forearm and three fasting blood samples for glucose and insulin were taken five minutes apart (-15, -10, -5 minutes). Participants then ingested (0 minutes) a 75 gram solution of dextrose and venous blood samples were obtained again at 30, 60, and 120 minutes for determination of plasma glucose and insulin. Glucose was measured by a glucose oxidase method and serum insulin was measured with an immunometric assay (Diagnostic Products Corporation, Los Angeles, CA). The glucose and insulin data from the OGTT was used to assess insulin sensitivity (homeostatic model assessment index,  $HOMA = \text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mmol/L)} \text{ divided by } 22.5$  (26)) and insulin secretion (insulinogenic index (ISI) = ratio of change in insulin to change in glucose from 0 to 30 minutes ( $\Delta I_{30}$  divided by  $\Delta G_{30}$ ) (27)). The HOMA index has been validated with the gold standard euglycemic hyperinsulinemic clamp technique (28). During the OGTT, fasting blood samples were also collected for serum total, HDL and LDL cholesterol, free fatty acids, and triglycerides. LDL was calculated using the Friedewald formula (29).

#### **5.4.6 Magnetic Resonance Imaging**

Intramyocellular lipid (IMCL) content of the mid-soleus muscle (predominately oxidative muscle fibres) was assessed by proton magnetic resonance spectroscopy ( $^1\text{H}$ -MRS) at the end of the run-in period. MRS was performed on a 1.5 Tesla whole body MR system (Siemens Symphony AG, Munich, Germany) using a body coil for radiofrequency transmission and surface coil to receive signals. The  $^1\text{H}$ -MRS scans were performed at the Nuclear Medicine Department at the McMaster University Medical Centre. Participants were advised to fast and restrict physical activity for six hours prior to the procedure. Following screening for absence of MRI contraindications, the participant was placed in the supine position and the leg was positioned and immobilized so that the calf was situated as close to the center of the magnet as possible. The radio-frequency receive coil was fastened nearest to the region of interest to collect the greatest signal. Participants remained in the supine position (feet first) within the MR system. Three-plane spin-echo T1-weighted MR images were performed to guide placement of the volume of interest for spectroscopy. Imaging parameters were chosen for suitable separation of muscle, fascia, IMCL and extramyocellular lipid (EMCL) content (TR 3000 milliseconds (ms), TE 30 ms, 128 averages, 1,024 data points over 1000 Hz spectral width, 1 cc voxel volume, water signal suppressed using chemical selective saturation). Semi-automatic shimming of the magnet with typical line widths of the water signal of 10 Hz was performed to optimize magnetic field homogeneity. Several scout images were taken to determine the ideal position for the voxel location. Volume of interest was centered over the mid-soleus muscle and vascular structures and gross adipose tissue deposits were excluded as much as possible. The IMCL and EMCL peak was integrated at 1.28 ppm and 1.48 ppm respectively. Since creatine content is stable within the same muscle across a population (50), spectral intensities were referenced to the

methyl signal of creatine ( $\text{Cr}_3$ ) at 3.05 ppm serving as an internal reference. Spectra were processed and the resonance curves for IMCL, EMCL, and creatine were measured using jMRUI software v2.1 (51). Data is presented as arbitrary units (AU).

After 3-plane localizer image acquisition, breath-hold axial T1-weighted image at the level of mid-L4 (TR 400 ms, TE 13 ms) was acquired for the volume of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). VAT and SAT was calculated using SliceOmatic 4.2 medical imaging software (SliceOmatic v.4.2, Tomovision, Montreal). VAT was defined as adipose tissue within the inside edge of the abdominal wall and SAT was defined as adipose tissue on the outside edge of the abdominal wall. The intra-and-inter-observer coefficients of variation for this method are 0.53% and 0.44% for SAT and 1.46% and 2.42% for VAT respectively.

#### **5.4.7 Statistical Analysis**

The statistical analyses were performed using SPSS 10.1 for Windows (SPSS Inc., Chicago, IL, USA). The primary analysis was an ANOVA of change in IMCL content in the soleus muscle by treatment group covarying out baseline IMCL measurement, age, sex, change in body weight, and body mass index. Pearson product moment correlation coefficient and Student's t-tests were used to determine correlations and differences of means between IMCL, GI, macronutrient composition of the diet, anthropometric data, blood glucose, blood insulin and blood lipid parameters. Statistical significance was set at  $p < 0.05$ . All data are presented as means  $\pm$  standard deviation (SD) unless otherwise indicated.

Assuming a two-sided alpha of 0.05 and a standard deviation of 5, the study sample was powered at 90% to detect a minimum difference in change in IMCL content between treatment groups of 3.7 (Arbitrary Units (AU)), based on a t-test with 40 participants per group.

Additional participants were randomized to account for an anticipated drop-out rate of 30%. The study sample was also powered at 90% to detect average changes between the start and end of the 24 week study for insulin sensitivity of 0.96, free fatty acids of 26.6  $\mu\text{mol/L}$ , triglycerides of 0.49 mmol/L, total cholesterol of 0.62 mmol/L, and LDL cholesterol of 0.56 mmol/L.

## **5.5 Results**

### **5.5.1 Subject Characteristics**

Entering the study, participants in the LGID and HGID group had abdominal obesity (100%) and were similar in age (LGID  $53.1 \pm 9.2$  vs HGID  $53.5 \pm 9.9$ ) and sex (female, LGID 71% vs HGID 70%). The majority of the participants were Caucasian (LGID 88% vs HGID 94%) and the women were menopausal (LGID 97% vs HGID 91%). Clinical characteristics of the study population at baseline and end-of-study are listed in Table 5.1.

### **5.5.2 Anthropometric Measurements**

BMI significantly increased from the start-to-end of the study in the HGID group ( $34.8 \pm 6.1$  vs  $35.0 \pm 6.2$ ,  $p < 0.05$ ), but did not significantly change in the LGID group ( $34.0 \pm 5.2$  vs  $33.9 \pm 5.4$ ,  $p > 0.05$ ). All participants in both groups maintained abdominal obesity throughout the study for both the LGID group (start  $109.5 \pm 14.0$  vs end  $110.0 \pm 14.4\text{cm}$ ) and the HGID group (start  $111.6 \pm 11.1$  vs end  $112.0 \pm 12.1\text{cm}$ ) but was not significant from start-to-end of study or between groups ( $p > 0.05$ ). WC, HC, WHR, and percentage body fat did not significantly change in either group, nor were there any significant differences between groups for any of the variables after controlling for baseline ( $p > 0.05$ ). The participants' anthropometric characteristics at baseline and end of study are shown in Table 5.2.



**Table 5.1.** Clinical Characteristics of the Study Population at Baseline and End of Study

	<b>LGID</b>	<b>LGID</b>	<b>HGID</b>	<b>HGID</b>
	<b>Baseline</b>	<b>End</b>	<b>Baseline</b>	<b>End</b>
<b>Variable</b>	<b>N = 48</b>	<b>N = 48</b>	<b>N = 47</b>	<b>N = 47</b>
Age	53.1 [ $\pm$ 9.2]		53.5 [ $\pm$ 9.9]	
Male	14 (29.2)		14 (29.8)	
Female	34 (70.8)		33 (70.2)	
Menopause	33 (97.1)		30 (90.9)	
Caucasian	42 (87.5)		44 (93.6)	
Smoker	18 (37.5)		16 (34.0)	
Calcium Channel Blocker medication	4 (9.1)	2 (4.7)	4 (8.9)	3 (6.7)
Anti-hypertensive medications	10 (20.8)	9 (19.6)	5 (10.6)	4 (8.5)
Diuretics	3 (6.3)	2 (4.3)	2 (4.3)	2 (4.3)
Aspirin	3 (6.3)	5 (11.4)	5 (10.6)	4 (8.9)
Lipid Lowering Drugs	9 (18.8)	10 (20.8)	7 (14.9)	7 (14.9)
Abdominal Obesity	48 (100)	48 (100)	47 (100)	47 (100)
Triglycerides $\geq$ 1.7 mmol/L	25 (52.1)	26 (60.5)	26 (55.3)	20 (45.5)
Blood Pressure $\geq$ 130/85 mmHg	13 (27.1)	11 (22.9)	10 (21.3)	8 (17.0)
Fasting Glucose 6.1- 6.9 mmol/L	7 (14.9)	8 (18.6)	7 (14.9)	7 (14.9)
HDL Cholesterol	15 (31.3)	20 (41.7)	16 (34.0)	21 (44.7)
<1.0 mmol/L (M), <1.3 mmol/L (F)				

Numbers in round brackets ( ) represent percentage of the study population. Numbers in square brackets [ ] represent  $\pm$ SD of the mean. M indicates males, F indicates females.

**Table 5.2.** Anthropometric and Body Composition Characteristics at Baseline and End of Study

	<b>LGID</b>	<b>LGID</b>		<b>HGID</b>	<b>HGID</b>	
	<b>Baseline</b>	<b>End</b>		<b>Baseline</b>	<b>End</b>	
<b>Variable</b>	<b>N = 48</b>	<b>N = 48</b>	<b>P value</b>	<b>N = 47</b>	<b>N = 47</b>	<b>p value</b>
BMI (kg/m <sup>2</sup> )	34.0±5.2	33.9±5.4	0.93	34.8±6.13	35.0±6.2	0.02*
WC (cm)	109.5±14.0	110.0±14.4	0.83	111.6±11.1	112.0±12.1	0.33
HC (cm)	120.2±10.7	119.4±11.7	0.61	122.1±12.6	121.9±11.4	0.68
WHR	0.91±0.08	0.92±0.07	0.37	0.92±0.07	0.92±0.07	0.62
% BF	41.2±10.9	41.4±7.3	0.99	41.7±9.0	41.9±8.8	0.41

Data are means ± SD, \*p < 0.05; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; % BF, percentage body fat

### 5.5.3 Blood Pressure and Heart Rate

Systolic BP significantly decreased in the HGID group (start 122±14 vs end 117±15mmHg, p<0.05) but not in the LGID group (start 117±11 vs end 116±16mmHg, p>0.05), nor were there any significant differences between groups after controlling for baseline (p>0.05). Diastolic BP and HR did not significantly change in either group or between groups (p>0.05).

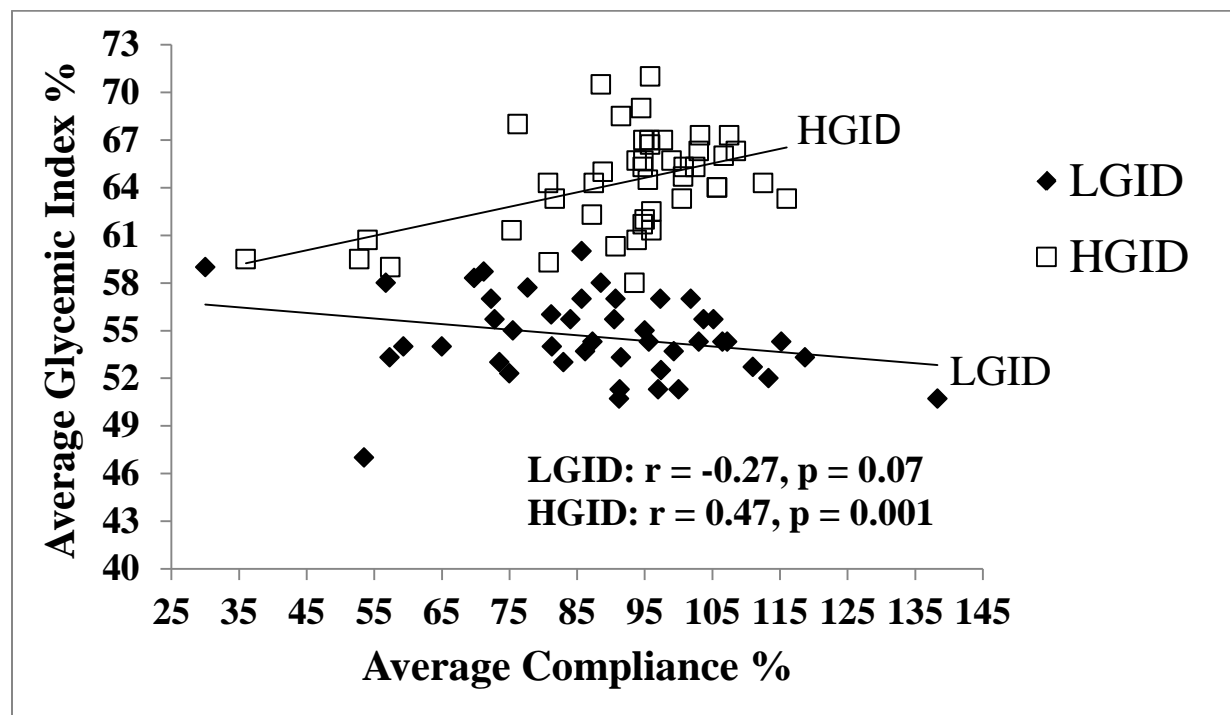
### 5.5.4 Dietary Intake and Physical Activity

#### 5.5.4.1 Activity Level

Activity level, as assessed by the Baecke questionnaire, did not significantly change during the randomization phase for either the LGID (p>0.05) (Table 5.3) or HGID (p>0.05) (Table 5.4) and was not significantly different between groups (p>0.05) (Table 5.5).

#### 5.5.4.2 Compliance

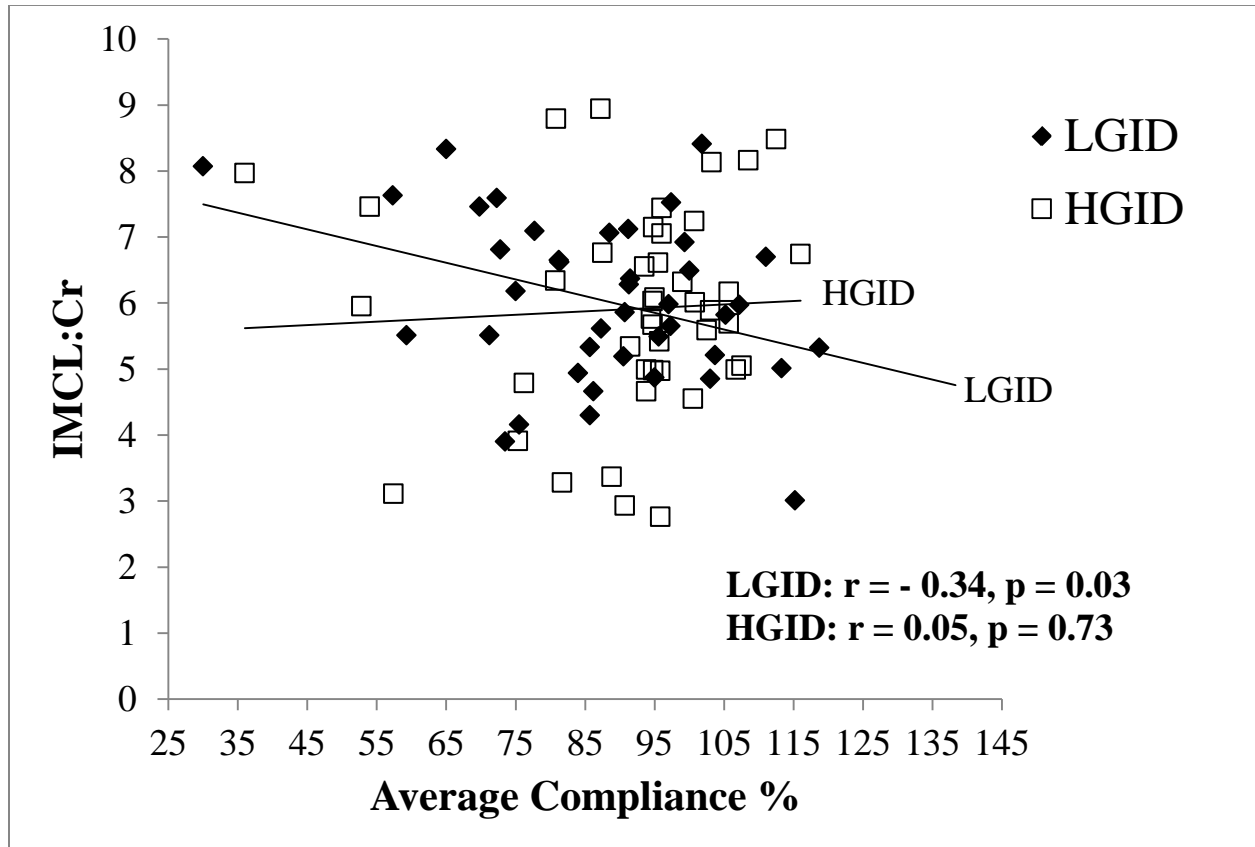
Self-reported data from the test food diaries indicated an average compliance for the consumption of test foods on the LGID was  $87.7\% \pm 19.7$  (range 30% to 138%) and  $91.6\% \pm 15.9$  (range 36% to 116%) on the HGID. Diet compliance was not significantly different between groups ( $p > 0.05$ ). There was a significant positive relationship between average compliance and average GI for the HGID ( $r = 0.47$ ,  $p = 0.001$ ) and a non-significant negative relationship between average compliance and average GI for the LGID ( $r = -0.27$ ,  $p = 0.07$ ) (Figure 5.2).



**Figure 5.2. Test Food Compliance and Glycemic Index**

Average compliance (%) of test foods from weekly test food diaries and mean GI for the high glycemic index diet, HGID □ (N = 47) and the low glycemic index diet, LGID ◆ (N = 48).

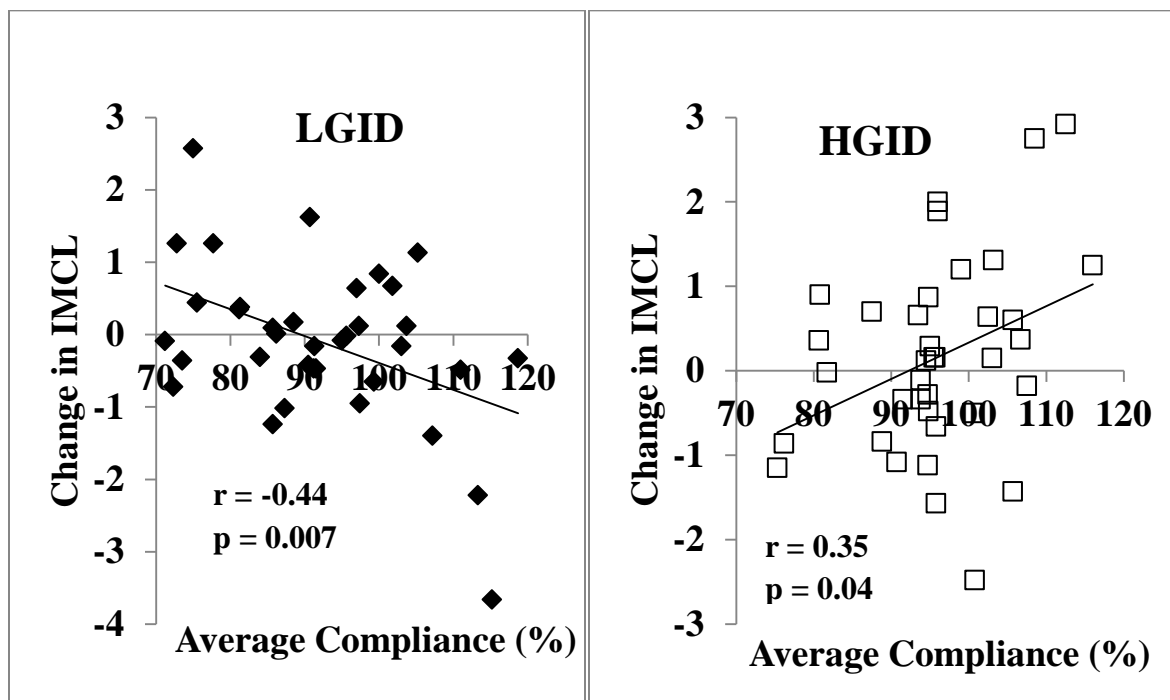
There was a significant negative correlation between average compliance of test foods and end of study IMCL for the LGID ( $r=-0.36$ ,  $p=0.02$ ) (Figure 5.3). No correlation was found between average compliance of test foods and end of study IMCL for the HGID ( $r=0.05$ ,  $p=0.73$ ) (Figure 5.3).



**Figure 5.3. Test Food Compliance and IMCL**

Average compliance (%) of test foods from weekly test food diaries (duration of study) and intramyocellular lipid content (IMCL) (end of study) for the high glycemic index diet, HGID □ (N = 40) and the low glycemic index diet, LGID ◆ (N = 40).

There was a significant negative correlation between average test food compliance for participants who were 70% or greater in compliance and change in IMCL from baseline to end of study for the LGID ( $r=-0.44$ ,  $p<0.007$ ). For the HGID, there was a significant positive correlation between average test food compliance (70% or greater) and change in IMCL from baseline to end of study ( $r=0.35$ ,  $p<0.04$ ) (Figure 5.4)



**Figure 5.4. Test Food Compliance of 70% or greater and Change in IMCL**

Average compliance (%) of test foods from weekly test food diaries for participants who were 70% or greater in compliance (duration of the study) and the change in intramyocellular lipid content (IMCL) from baseline to end of study for the low glycemic index diet, LGID ◆ (N = 36), and the HGID □ (N = 37).

#### 5.5.4.3 Dietary Intake

The 3-day average GI and macronutrient data from the self-reported food diaries at baseline and end-of-study for the LGID and HGID are listed in Table 5.3 and Table 5.4 respectively and comparisons between groups after controlling for baseline are listed in Table 5.5.

The participant's achieved a significant reduction in the GI on the LGID ( $59.6 \pm 4.0$  baseline vs  $55.4 \pm 2.7$  end,  $p=0.0000$ ) and a significant increase in the GI on the HGID ( $60.0 \pm 3.4$  baseline vs end  $63.9 \pm 3.5$ ,  $p=0.0000$ ). There was also a significant difference in the GI between groups after controlling for baseline (LGID  $55.5 \pm 3.1$  vs HGID  $63.9 \pm 3.1$ ,  $p<0.0001$ ). The average GI analyzed from the three-3 day food diaries on the LGID was  $54.6 \pm 2.6$  and  $64.3 \pm 3.1$  on the HGID and was significantly different between groups ( $p<0.0000$ ). The GI was not correlated with any anthropometric measurements, fasting blood lipids, or blood glucose and insulin measurements obtained from the OGTT ( $p>0.05$ ).

Energy intake (kilocalories (kcal)) significantly increased on the HGID ( $1980 \pm 626$  vs  $2212 \pm 524$  kcal,  $p<0.05$ ) but did not change on the LGID ( $2061 \pm 544$  vs  $2070 \pm 423$  kcal,  $p>0.05$ ). There were no significant differences in means between groups for energy intake after controlling for baseline (HGID  $2212 \pm 400$  vs LGID  $2065 \pm 400$  kcal,  $p>0.05$ ).

Total carbohydrate intake significantly increased on both the LGID (baseline  $261 \pm 92$  vs end  $316 \pm 75$  g,  $p<0.0001$ ) and the HGID (baseline  $259 \pm 86$  vs end  $304 \pm 69$  g,  $p<0.01$ ), but was not significant between groups after controlling for baseline (LGID  $316 \pm 62$  vs  $303 \pm 62$  g,  $p>0.05$ ). When expressed as a percentage of energy, carbohydrate significantly increased on the LGID (LGID baseline  $50 \pm 9$  vs end  $57 \pm 8\%$   $p<0.0001$ ) but not on the HGID (baseline  $52 \pm 8$  vs end

54±8%,  $p>0.05$ ), and was significantly different between groups after controlling for baseline (LGID 57±7 vs HGID 54±7%,  $p<0.05$ ).

Total dietary fibre intake significantly increased on the LGID (baseline 23±11 vs end 42±14g,  $p<0.0000$ ) but not on the HGID (baseline 25±12 vs end 24±7g,  $p>0.05$ ), and was significantly different between groups after controlling for baseline (LGID 43±10 vs HGID 23±10g  $p<0.0001$ ).

Total fat intake significantly decreased on the LGID (baseline 75±39 vs end 58±18g,  $p<0.05$ ) but not on the HGID (baseline 66±31 vs end 70±30g,  $p>0.05$ ) and was significantly different between groups after controlling for baseline (LGID 58±23 vs HGID 70±23g,  $p<0.05$ ). When expressed as a percentage of energy, total fat intake significantly decreased on the LGID (baseline 30±9 vs end 24±6,  $p<0.001$ ) but not on the HGID (baseline 29±6 vs end 27±7,  $p>0.05$ ) and was significantly different between groups after controlling for baseline (LGID 24±6 vs HGID 27±6,  $p<0.05$ ).

Saturated fat intake significantly decreased on the LGID (baseline 23±11 vs end 17±6g,  $p<0.01$ ) but not on the HGID (baseline 20±9 vs end 21±11g,  $p>0.05$ ), and was significantly different between groups after controlling for baseline (LGID 17±9 vs HGID 21±9g,  $p<0.05$ ). When expressed as a percentage of energy, saturated fat intake significantly decreased on the LGID (baseline 10±4. vs end 7±3%,  $p<0.001$ ) and decreased on the HGID but it was not significant (baseline 9±2 vs end 8±3%,  $p>0.05$ ).

Protein intake expressed as grams or percentage of energy and alcohol intake as a percentage of energy did not significantly change in either group or between groups after controlling for baseline ( $p>0.05$ ).

**Table 5.3.** Dietary Intake and Physical Activity at Baseline and End-of-Study for the LGID

	<b>Low GI</b>	<b>Low GI</b>	
	<b>Baseline</b>	<b>End</b>	
<b>Variable</b>	<b>N = 48</b>	<b>N = 48</b>	<b>p value</b>
GI (%)	59.6 $\pm$ 4.0	55.4 $\pm$ 2.7	<0.0000
Energy (kcal)	2061 $\pm$ 544	2070 $\pm$ 423	0.253
CHO (g)	261.3 $\pm$ 92.0	316.1 $\pm$ 74.7	<0.0001
CHO (% energy)	49.7 $\pm$ 9.4	57.3 $\pm$ 7.6	<0.0001
Fibre (g)	22.9 $\pm$ 11.0	42.0 $\pm$ 13.9	<0.0000
Protein (g)	93.0 $\pm$ 22.1	92.8 $\pm$ 23.6	0.384
Protein (% energy)	18.0 $\pm$ 3.4	17.1 $\pm$ 3.1	0.121
Fat (g)	75.0 $\pm$ 39.0	58.1 $\pm$ 18.0	<0.05
Fat (% energy)	30.3 $\pm$ 8.8	24.0 $\pm$ 6.3	<0.001
Saturated Fat (g)	22.7 $\pm$ 10.8	16.8 $\pm$ 6.0	<0.01
Saturated Fat (% energy)	10.0 $\pm$ 4.2	7.1 $\pm$ 2.5	< 0.001
Alcohol (% energy)	1.6 $\pm$ 4.0	1.3 $\pm$ 2.6	0.756
MEDFICTS score	47.2 $\pm$ 19.0	44.5 $\pm$ 16.3	0.551
Activity Level (Baecke)	2.4 $\pm$ 0.5	2.4 $\pm$ 0.8	0.700

Data are means  $\pm$  SD; GI, glycemic index; CHO, carbohydrate



**Table 5.4.** Dietary Intake and Physical Activity at Baseline and End-of-Study for the HGID

	<b>HGID</b>	<b>HGID</b>	
	<b>Baseline</b>	<b>End</b>	
<b>Variable</b>	<b>N = 47</b>	<b>N = 47</b>	<b>p value</b>
GI (%)	60.0 $\pm$ 3.4	63.9 $\pm$ 3.5	<0.0000
Energy (kcal)	1980 $\pm$ 626	2212 $\pm$ 524	<0.05
CHO (g)	258.9 $\pm$ 85.9	303.7 $\pm$ 69.3	<0.01
CHO (% energy)	52.3 $\pm$ 7.5	54.2 $\pm$ 7.6	0.105
Fibre (g)	24.6 $\pm$ 11.7	23.8 $\pm$ 7.0	0.553
Protein (g)	89.5 $\pm$ 36.1	99.3 $\pm$ 27.7	0.076
Protein (% energy)	18.0 $\pm$ 3.5	17.7 $\pm$ 3.1	0.202
Fat (g)	66.4 $\pm$ 30.5	69.9 $\pm$ 29.6	0.494
Fat (% energy)	28.9 $\pm$ 6.0	27.2 $\pm$ 6.8	0.208
Saturated Fat (g)	20.3 $\pm$ 8.5	21.0 $\pm$ 11.3	0.575
Saturated Fat (% energy)	9.0 $\pm$ 2.2	8.2 $\pm$ 2.9	0.141
Alcohol (% energy)	0.9 $\pm$ 2.0	1.6 $\pm$ 4.3	0.169
MEDFICTS score	44.7 $\pm$ 18.2	44.3 $\pm$ 14.4	0.970
Activity Level (Baecke)	2.6 $\pm$ 0.5	2.4 $\pm$ 0.8	0.118

Data are means  $\pm$  SD; GI, glycemic index; CHO, carbohydrate

**Table 5.5.** Dietary Intake and Physical Activity for the LGID vs the HGID after Controlling for Baseline

	<b>LGID</b>	<b>HGID</b>	
<b>Variable</b>	<b>N = 48</b>	<b>N = 47</b>	<b>p value</b>
Glycemic Index (%)	55.5 $\pm$ 3.1	63.9 $\pm$ 3.1	<0.0001
Energy (kcal)	2064.7 $\pm$ 400.1	2211.8 $\pm$ 400.1	0.107
Carbohydrate (g)	316.1 $\pm$ 62.0	303.1 $\pm$ 62.0	0.353
Carbohydrate (% energy)	57.5 $\pm$ 7.1	54.0 $\pm$ 7.1	<0.05
Fibre (g)	42.6 $\pm$ 10.2	23.3 $\pm$ 10.2	<0.0001
Protein (g)	93.3 $\pm$ 22.1	98.9 $\pm$ 22.1	0.267
Protein (% energy)	17.2 $\pm$ 2.9	17.6 $\pm$ 2.9	0.524
Fat (g)	57.8 $\pm$ 23.0	70.2 $\pm$ 23.0	<0.05
Fat (% energy)	23.9 $\pm$ 6.4	27.3 $\pm$ 6.4	<0.05
Saturated Fat (g)	16.7 $\pm$ 8.7	21.2 $\pm$ 8.7	<0.05
Saturated Fat (% energy)	7.1 $\pm$ 2.6	8.3 $\pm$ 2.6	0.058
Alcohol (% energy)	1.0 $\pm$ 2.9	1.8 $\pm$ 2.9	0.262
MEDFICTS score	44.3 $\pm$ 14.6	44.6 $\pm$ 14.6	0.920
Activity Level (Baecke)	2.4 $\pm$ 0.7	2.4 $\pm$ 0.7	0.620

Data are means  $\pm$  SD; data are differences in means controlling for baseline

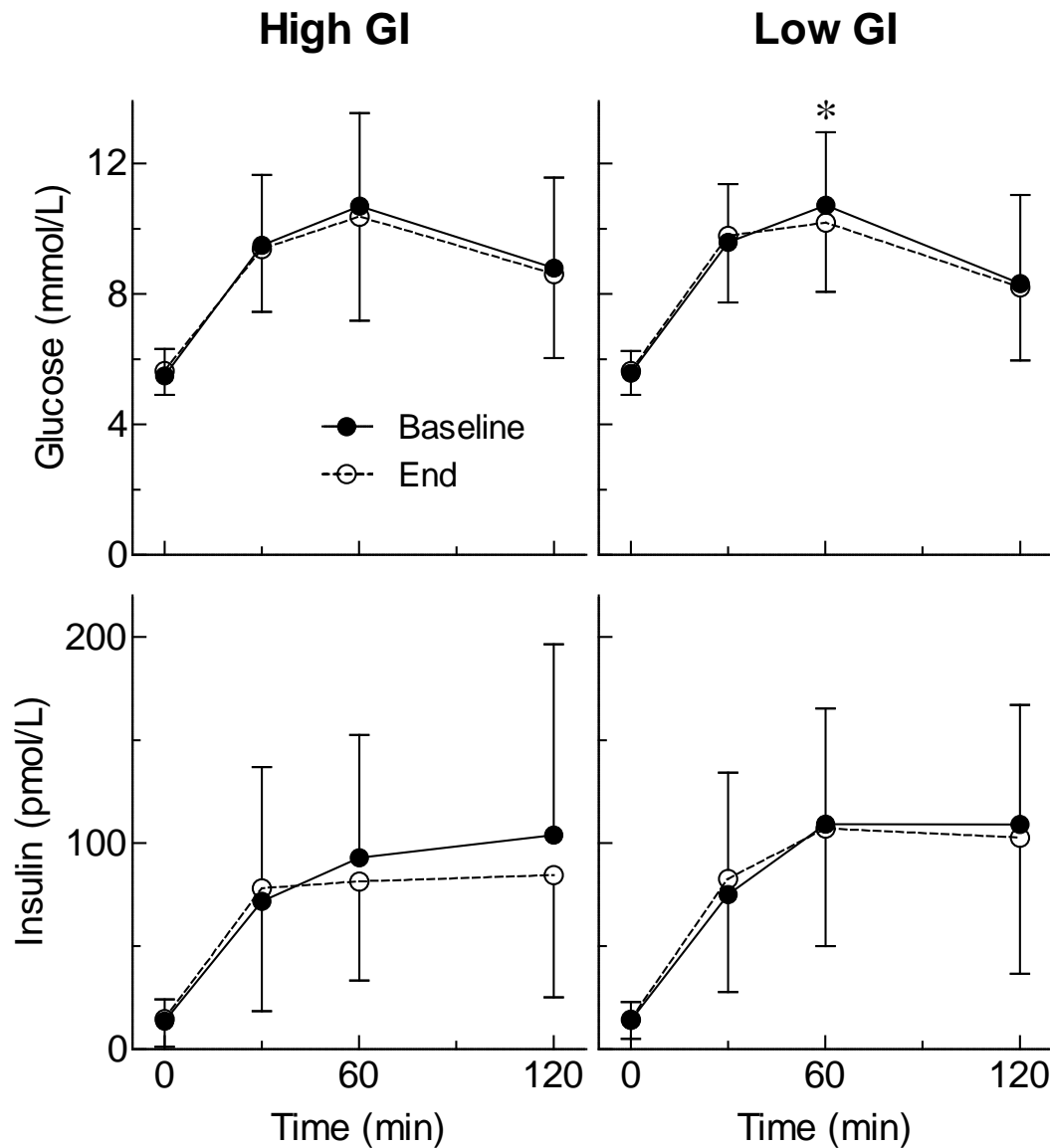
## 5.5.5 Oral Glucose Tolerance Test

### 5.5.5.1 Insulin and Glucose

HOMA index, fasting insulin, and insulin at 30, 60, and 120 minutes during the OGTT, did not significantly change in either group or between groups after controlling for baseline ( $p>0.05$ ) (Figure 5.4, Table 5.6). The insulinogenic index (ISI) significantly increased in the HGID group (baseline  $15.2\pm9.7$  vs end  $19.2\pm15.0$ ,  $p<0.01$ ) and there was a non-significant trend towards an increase in ISI in the LGID group (baseline  $16.2\pm10.9$  vs end  $19.5\pm15.9$ ,  $p=0.07$ ) (Table 5.6). The incremental area under the curve for insulin (iiAUC) significantly decreased in the HGID (baseline  $7047.0\pm4661.8$  vs end  $6718.2\pm4322.6$ ,  $p<0.05$ ) and a non-significant decrease in the iiAUC in the LGID (baseline  $6858.9\pm5500.1$  vs end  $6622.6\pm5658.2$ ,  $p>0.05$ ) (Table 5.6). There was also a significant positive correlation between baseline and end-of-study iiAUC for the LGID ( $r=0.77$ ,  $p<0.0001$ ) and the HGID ( $r=0.89$ ,  $p<0.0001$ ) but the slope of the regression lines were not significantly different between groups ( $p>0.05$ ) (Figure 5.5).

During the OGTT, 60 minute plasma glucose significantly decreased in the LGID (baseline  $10.7\pm2.2$  vs end  $10.2\pm2.1$ ,  $p<0.05$ ), but not in the HGID (baseline  $10.7\pm2.9$  vs end  $10.4\pm3.2$ ,  $p>0.05$ ) and fasting, 30 minute and 120 minute plasma glucose did not significantly change in either group ( $p>0.05$ ) (Figure 5.4, Table 5.6). There was a significant positive correlation between baseline and end-of-study 120 minute plasma glucose for the LGID ( $r=0.62$ ,  $p<0.0001$ ) and the HGID ( $r=0.81$ ,  $p<0.0001$ ) and the regression slopes significantly differed between the LGID and the HGID ( $p=0.04$ ) (Figure 5.6). The incremental area under the curve for glucose (igAUC) significantly decreased in the HGID (baseline  $461.6\pm171.2$  vs end  $423.8\pm185.3$ ,  $p<0.05$ ) and decreased in the LGID but was not significant (baseline  $440.3\pm153.6$  vs end  $399.9\pm138.1$ ,  $p>0.05$ ) (Figure 5.4, Table 5.6). There was a significant positive correlation

between baseline and end-of-study igAUC for the LGID ( $r=0.56$ ,  $p<0.001$ ) and the HGID ( $r=0.80$ ,  $p<0.0001$ ), and the slopes of the regression lines were significantly lower on the LGID compared to the HGID ( $p=0.04$ ) (Figure 5.5).

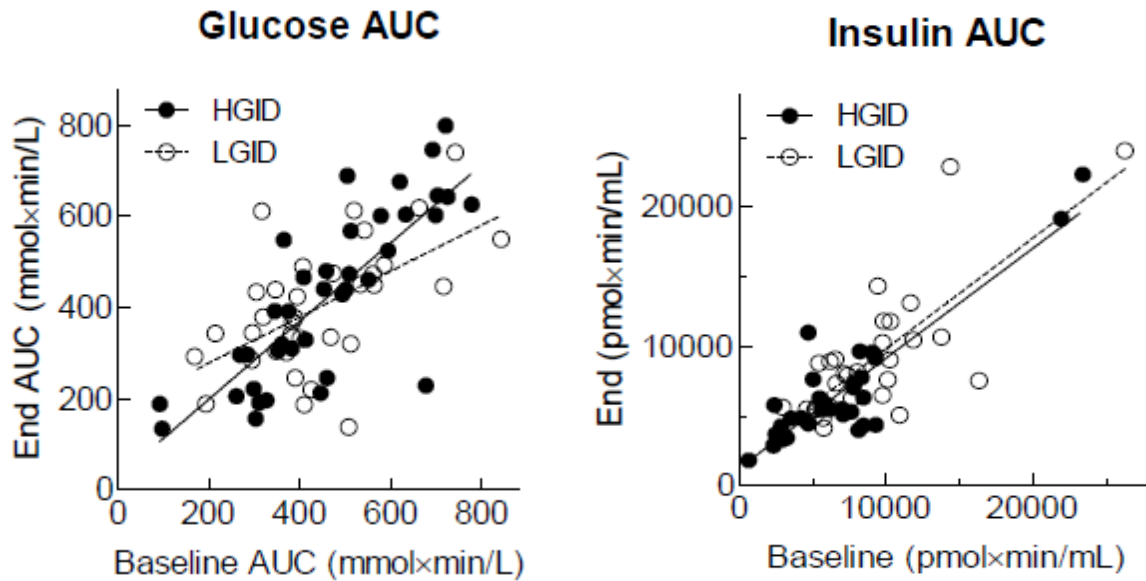


**Figure 5.4.** Mean Glucose and Insulin Values at Baseline (•) and End-of-Study (o) for the HGID and LGID, obtained during the OGTT. Plasma glucose at 60 minutes significantly decreased in the LGID group (\* $p<0.05$ ).

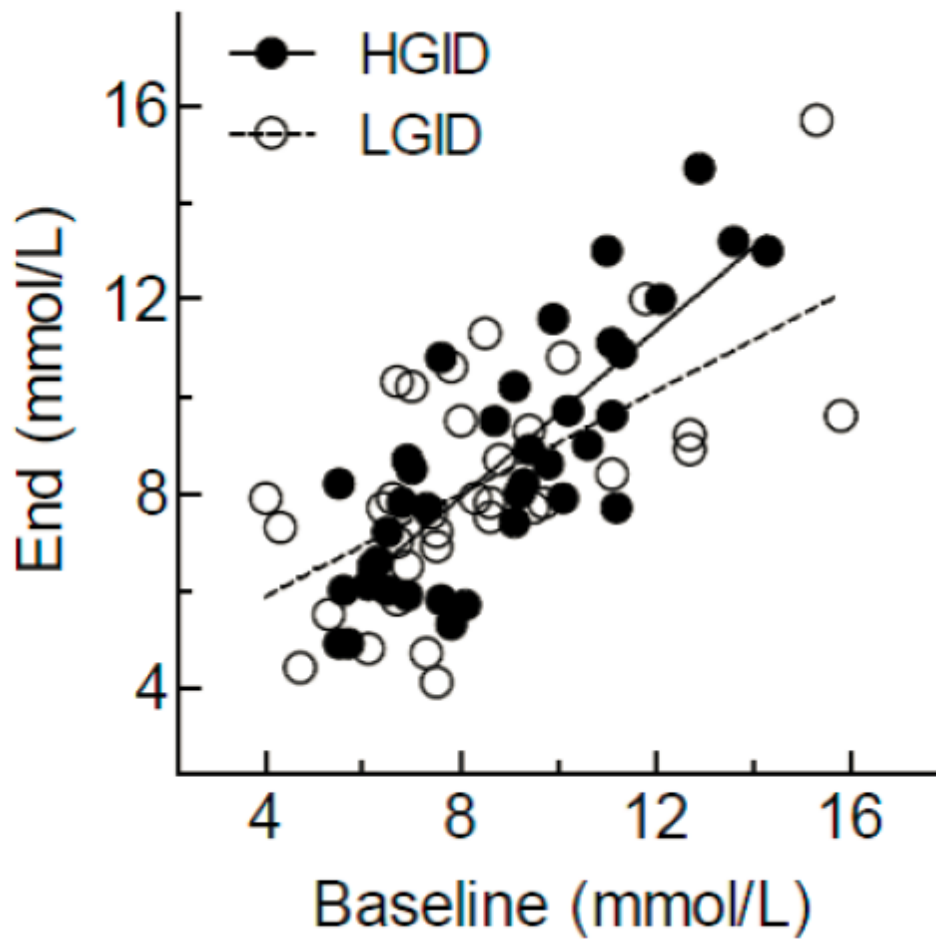
**Table 5.6.** Fasting Plasma Glucose and Insulin at Baseline and End of Study

	<b>LGID</b>	<b>LGID</b>		<b>HGID</b>	<b>HGID</b>	
	<b>Baseline</b>	<b>End</b>	<b>p</b>	<b>Baseline</b>	<b>End</b>	<b>p</b>
<b>Variable</b>	<b>N = 48</b>	<b>N = 48</b>	<b>value</b>	<b>N = 47</b>	<b>N = 47</b>	<b>value</b>
HOMA	3.6 $\pm$ 2.4	3.7 $\pm$ 2.3	0.72	3.6 $\pm$ 4.8	3.7 $\pm$ 2.5	0.94
ISI	16.2 $\pm$ 10.9	19.5 $\pm$ 15.9	0.07	15.2 $\pm$ 9.7	19.2 $\pm$ 15.0	0.00**
<b>Glucose</b>						
Fasting	5.6 $\pm$ 0.7	5.6 $\pm$ 0.6	0.52	5.5 $\pm$ 0.8	5.6 $\pm$ 0.7	0.17
30 min	9.6 $\pm$ 1.8	9.8 $\pm$ 1.6	0.93	9.5 $\pm$ 2.2	9.4 $\pm$ 1.9	0.29
60 min	10.7 $\pm$ 2.2	10.2 $\pm$ 2.1	0.03*	10.7 $\pm$ 2.9	10.4 $\pm$ 3.2	0.07
120 min	8.3 $\pm$ 2.7	8.2 $\pm$ 0.172	0.17	8.8 $\pm$ 2.8	8.6 $\pm$ 2.6	0.89
igAUC	440.3 $\pm$ 153.6	399.9 $\pm$ 138.1	0.10	461.6 $\pm$ 171.2	423.8 $\pm$ 185.3	0.05*
<b>Insulin</b>						
Fasting	13.9 $\pm$ 8.9	14.3 $\pm$ 8.6	0.66	13.7 $\pm$ 12.5	14.6 $\pm$ 9.5	0.58
30 min	75.1 $\pm$ 47.4	82.5 $\pm$ 51.6	0.33	71.2 $\pm$ 53.3	78.1 $\pm$ 58.7	0.25
60 min	109.2 $\pm$ 56.0	106.7 $\pm$ 57.0	0.83	92.8 $\pm$ 59.7	81.3 $\pm$ 48.1	0.78
120 min	108.9 $\pm$ 58.1	102.6 $\pm$ 66.0	0.84	103.9 $\pm$ 92.6	84.5 $\pm$ 59.3	0.31
iiAUC	6858.9	6622.6	0.50	7047.0	6718.2	0.02*
	$\pm$ 5500.1	$\pm$ 5658.2		$\pm$ 4661.8	$\pm$ 4322.6	

Data are means  $\pm$  SD; ISI, insulinogenic index; plasma glucose is measured in mmol/L; plasma insulin is measured in pmol/L; igAUC, incremental area under the curve for plasma glucose obtained during the OGTT; iiAUC, incremental area under the curve for plasma insulin obtained during the OGTT; \*p < 0.05, \*\*p < 0.01



**Figure 5.5.** Relationship between Baseline and End-of-Study Incremental Area under the Plasma Glucose Curve (Glucose AUC) and Incremental Area under the Plasma Insulin Curve (Insulin AUC) Obtained During the OGTT for the HGID (●) and LGID (○). For glucose AUC, correlations were statistically significant for both HGID ( $r=0.795$ ,  $p<0.0001$ ) and LGID ( $r=0.562$ ,  $p<0.001$ ), and the difference in the regression slopes was significantly different ( $p=0.04$ ). For insulin AUC, correlations were statistically significant for both HGID ( $r=0.886$ ,  $p<0.0001$ ) and LGID ( $r=0.769$ ,  $p<0.0001$ ), but there was no significant difference in the regression slopes between the two groups ( $p>0.05$ ).



**Figure 5.6.** Relationship between Baseline and End-of-Study 120 minute Plasma Glucose obtained during the OGTT for the HGID (●) and LGID (○). Correlations were statistically significant for both HGID ( $r=0.81$ ,  $p<0.0001$ ) and LGID ( $r=0.62$ ,  $p<0.0001$ ) and the differences in slopes between the two groups was significant ( $p=0.04$ ).

### 5.5.5.2 Blood Lipids

There were no significant changes in fasting total serum cholesterol, LDL cholesterol, HDL cholesterol, triglycerides or free fatty acids in either group, nor were there any significant differences between groups after controlling for baseline ( $p > 0.05$ ). The participants' fasting serum lipids at baseline and end of study are shown in Table 5.7.

**Table 5.7.** Fasting Serum Lipids at Baseline and End of Study

	<b>LGID</b>			<b>HGID</b>		
	<b>Baseline</b>	<b>End</b>	<b>p</b>	<b>Baseline</b>	<b>End</b>	<b>p</b>
<b>Variable</b>	<b>N = 48</b>	<b>N = 48</b>	<b>value</b>	<b>N = 47</b>	<b>N = 47</b>	<b>Value</b>
Total C (mmol/L)	5.3 $\pm$ 1.1	5.2 $\pm$ 1.1	0.31	4.9 $\pm$ 0.9	4.9 $\pm$ 0.8	0.78
LDL (mmol/L)	3.1 $\pm$ 0.9	3.0 $\pm$ 0.9	0.16	2.8 $\pm$ 0.8	2.8 $\pm$ 0.7	0.58
HDL (mmol/L)	1.3 $\pm$ 0.4	1.3 $\pm$ 0.4	0.28	1.2 $\pm$ 0.3	1.2 $\pm$ 0.4	0.24
TG (mmol/L)	1.9 $\pm$ 0.8	2.1 $\pm$ 0.9	0.15	2.2 $\pm$ 1.7	2.1 $\pm$ 1.4	0.63
FFA ( $\mu$ mol/L)	594.2	600.9	0.73	635.4	566.3	0.07
	$\pm$ 231.9	$\pm$ 228.2		$\pm$ 235.6	$\pm$ 208.0	

Data are means  $\pm$  SD; C, cholesterol; TG, triglycerides; FFA, free fatty acids

### 5.5.6. Intramyocellular Lipids (IMCL), Subcutaneous Adipose Tissue (SAT), Visceral Adipose Tissue (VAT)

There were no significant changes in IMCL, in either group during the dietary intervention or between groups after controlling for baseline. There were no significant changes in either group during the dietary intervention or between groups for SAT or VAT after controlling for



baseline ( $p>0.05$ ). The participants IMCL, VAT and SAT at baseline and end of study are shown in Table 5.8.

Post dietary intervention, there were significant positive correlations with IMCL and BMI ( $r=0.21$ ,  $p<0.5$ ), WC ( $r=0.33$ ,  $p<0.01$ ), WHR ( $r=0.37$ ,  $p<0.001$ ), VAT ( $r=0.34$ ,  $p<0.01$ ), HOMA ( $r=0.23$ ,  $p<0.05$ ), fasting plasma insulin ( $r=0.26$ ,  $p<0.05$ ), diastolic BP ( $r=0.29$ ,  $p<0.01$ ), percentage of energy from saturated fat ( $r=0.23$ ,  $p<0.05$ ) and total fat intake (g) ( $r=0.29$ ,  $p<0.05$ ). IMCL was not significantly correlated with GI ( $p>0.05$ ).

**Table 5.8.** IMCL, VAT and SAT at Baseline and End of Study

	<b>LGID</b>			<b>HGID</b>		
	<b>Baseline</b>	<b>End</b>	<b>p</b>	<b>Baseline</b>	<b>End</b>	<b>p</b>
<b>Variable</b>	<b>N = 40</b>	<b>N = 40</b>	<b>value</b>	<b>N = 40</b>	<b>N = 40</b>	<b>value</b>
IMCL:Cr	6.1 $\pm$ 1.3	6.1 $\pm$ 1.2	0.52	5.7 $\pm$ 1.2	5.9 $\pm$ 1.6	0.42

	<b>LGID</b>			<b>HGID</b>		
	<b>Baseline</b>	<b>End</b>	<b>p</b>	<b>Baseline</b>	<b>End</b>	<b>p</b>
<b>Variable</b>	<b>N = 48</b>	<b>N = 48</b>	<b>value</b>	<b>N = 47</b>	<b>N = 47</b>	<b>value</b>
VAT	211.4 $\pm$ 73.6	226.7 $\pm$ 86.5	0.16	195.8 $\pm$ 63.4	201.9 $\pm$ 75.0	0.53
SAT	365.0 $\pm$ 125.4	373.7 $\pm$ 125.4	0.24	405.0 $\pm$ 148.9	388.0 $\pm$ 148.5	0.82

Data are means  $\pm$  SD; muscle triglycerides are expressed as intramyocellular lipid:total creatine ratio (IMCL/Cr) (Arbitrary Units (AU)); VAT, visceral adipose tissue (AU); SAT, subcutaneous adipose tissue (AU).

## 5.6 Discussion and Conclusions

Individuals who are obese or have abdominal obesity tend to consume excess kcal and/or fat, which results in an accumulation of fat in organs other than adipose tissue such as muscle causing insulin resistance in these tissues (13, 14). Research has demonstrated that dietary fat intake is associated with IMCL content (30, 31). This study found significant positive correlations with IMCL and total dietary fat (g) and percentage of energy from saturated fat ( $p < 0.05$ ) which supports the role of IMCL being a storage site for fat when dietary fat is increased. Further, IMCL was positively correlated with the HOMA index at end of study ( $r = 0.23$ ,  $p < 0.05$ ) supporting the research demonstrating the positive relationship between IMCL and insulin resistance (15, 16).

Insulin resistance is higher in individuals with abdominal obesity (10,11) and puts them at risk for developing type 2 diabetes, coronary heart disease, and metabolic syndrome (2, 3, 4, 5, 6, 7, 8,12). de Koning et al (32) studied various ethnic groups in 5 different countries who were at risk of developing type 2 diabetes and found that an increase in BMI, WC and WHR all had positive associations with type 2 diabetes. This study showed significant positive correlations with IMCL and BMI ( $p < 0.05$ ), WC ( $p < 0.01$ ) and WHR ( $p < 0.001$ ) which supports the beneficial effects of reducing IMCL in a population at risk for developing type 2 diabetes. This study also found positive linear correlations with GI and BMI, WC, and WHR but they were not significant post intervention ( $r = 0.21$ ,  $p = 0.06$ ;  $r = 0.20$ ,  $p = 0.07$ ;  $r = 0.17$ ,  $p = 0.13$  respectively). Liese et al (33) found similar results and suggested that the lack of relationship between GI and adiposity in their study may have been due to an average GI of  $58.0 \pm 4.0$  which is similar to high GI diets in other studies (18). In contrast to these findings, Ma et al (34) found the GI was independently and positively associated with BMI ( $p = 0.01$ ), and the mean GI was also  $58.0 \pm 3.9$ . This study had a

slightly lower average GI of  $55.4 \pm 2.7$  for the LGID post-intervention. The difference in results may be due to the difference in ranges for BMI. Ma et al (34) collected data on individuals with a BMI varying from 18.5 to  $\geq 30$ , whereas this study only looked at abdominally obese individuals (BMI  $34.4 \pm 5.7$ ).

This study investigated whether a 24 week low glycemic index diet can improve insulin sensitivity by reducing IMCL content of skeletal muscle in weight stable abdominally obese individuals. Despite a significant reduction in GI on the LGID ( $59.6 \pm 4.0$  baseline vs end  $55.4 \pm 2.7$ ,  $p=0.0000$ ) and a significant increase in the GI on the HGID ( $60.0 \pm 3$  baseline vs end  $63.9 \pm 3.5$ ,  $p=0.0000$ ), as well as a significant difference in the GI between groups after controlling for baseline (LGID  $55.5 \pm 3.1$  vs HGID  $63.9 \pm 3.1$ ,  $p<0.0001$ ), neither IMCL or insulin resistance (HOMA) significantly changed on the LGID ( $p>0.05$ ). There was a trend towards an improvement in insulin secretion (insulinogenic index (ISI)) on the LGID but it was not significant ( $p=0.07$ ), however the HGID did significantly improve ISI ( $p<0.01$ ). The significant improvement in ISI in the HGID is not consistent with other research, especially since the HGID group significantly increased BMI ( $p<0.05$ ) due to a significant increase in total daily energy intake ( $p<0.05$ ). Research generally favours weight loss for improvements in insulin sensitivity without reductions in IMCL. Larson-Meyer et al (35) examined the effect of weight loss and exercise on IMCL and insulin sensitivity and found no reductions in IMCL but improvements in insulin sensitivity in all intervention groups (25% kcal restriction, 12.5% kcal reduction and 12.5% increase in energy expenditure, kcal restriction until a 15% reduction in body weight followed by weight maintenance) ( $p<0.05$ ), however there was no significant improvements in insulin sensitivity between groups.

This study does not support the hypothesis that a weight maintaining LGID will improve insulin sensitivity by reducing IMCL content of skeletal muscle in abdominally obese individuals. The lack of relationship may be due to the minimal change of -6.9% in GI for the LGID. Goff et al (20) also investigated the effects of a 4 week low GI diet on IMCL and also found no changes in storage levels of IMCL which may have been attributed to only a 15% change in GI. Goff et al (20) did find significant changes in insulin sensitivity following the low GI diet suggesting that insulin sensitivity may be independent of IMCL storage.

Another possible explanation for the lack of relationship between GI and IMCL could be due to diet compliance. A limitation of the present study was the inability to control compliance of the self-selected low or high GI test foods that the participants were required to consume each day. Participants were free-living and choose a specific number of test foods from a list based on their estimated energy intake and recorded the food choices on a daily food diary. The compliance was calculated from self-reported data on the food diaries at the monthly visit. A criticism of the low GI diet is that it is too complicated and difficult to follow (42). This study does not support this criticism since average diet compliance for the LGID was high ( $87.7 \pm 19.7\%$ ), however it is evident that not all individuals are willing to make changes to their diet since compliance for the LGID ranged from 30% to 138%. Similar results were found for the HGID; average diet compliance was  $91.6 \pm 15.9\%$  and ranged from 36% to 116%. These results demonstrate that it is difficult to follow any type of diet if individuals are not motivated to make changes to their usual dietary habits. Further, results showed a relationship between average GI and average diet compliance for both the LGID and HGID. As diet compliance increased on the LGID, the GI decreased ( $p=0.07$ ), and as diet compliance increased on the HGID, the GI increased ( $p<0.05$ ). For example, the lowest compliance of 30% on the LGID

resulted in an average GI of 59 which is similar to the average GI of 59.5 for the participant who was 36% compliant on the HGID. For the participants who were highest in compliance (LGID 138% and HGID 116%), there was a greater difference in the GI of the diets (LGID 50.7 vs HGID 63.3), which further demonstrates the importance of diet compliance. Results also showed a significant ( $p<0.05$ ) negative correlation between average diet compliance and IMCL for the LGID, indicating that as diet compliance increases, IMCL decreases on the LGID. In considering future long term studies, the compliance issue may need to be addressed to find significant differences in outcome variables.

It is well documented that individuals with abdominal obesity are at risk for developing diabetes (36), cardiovascular disease (36) and metabolic syndrome (7, 8). One characteristic of metabolic syndrome is fasting blood glucose of  $\geq 6.1$  mmol/L (1). Fasting blood glucose values of 6.1 to 6.9 is termed impaired fasting glucose (IFG) (37), or commonly called “prediabetes”, which is also a risk factor for developing diabetes and cardiovascular disease (38). Maintaining and/or improving glycemic control is therefore an important goal for abdominally obese individuals, a population at risk for developing type 2 diabetes and cardiovascular disease. Although only 14.6% of the participants entering this study had a fasting blood glucose of  $\geq 6.1$  mmol/L, the LGID did show significant improvements in 60 minute blood glucose obtained from the OGTT ( $p<0.05$ ) indicating that the responses to the different diets varied significantly post intervention. Further, the slope of the regression line was significantly lower for the LGID compared to the HGID for 120 minute glucose obtained from the OGTT ( $p = 0.04$ ). This suggests that the LGID lowers 120 minute glucose in abdominally obese individuals if their blood glucose is high to begin with.

Interventional studies examining the effects of dietary GI on serum lipids tend to favour the role of the GI in improving triglycerides, LDL cholesterol and HDL cholesterol (39, 40, 41). In the present study, despite the significant reductions in GI ( $p < 0.0001$ ), percentage of kcal from total fat ( $p < 0.001$ ) and saturated fat in the diet ( $p < 0.001$ ), there were no significant changes in fasting blood lipids post-intervention. A possible explanation may be that the participants reduced their fasting blood lipids to normal levels after completion of the 4-to-6 week low fat dietary advice run-in phase prior to entering this primary study. Participants in the run-in phase did significantly decrease fasting serum total cholesterol, LDL cholesterol and triglycerides ( $p < 0.0001$ ) due to a significant reduction in total dietary fat ( $p < 0.0001$ ) and saturated fat ( $p < 0.01$ ).

In conclusion, results from this study did not show improvements in insulin sensitivity or reduce IMCL content of skeletal muscle in weight-stable abdominally obese individuals after following a 24 week low glycemic index diet. Further research on the GI and IMCL is needed to determine whether these findings would occur in different populations such as those with impaired glucose tolerance or type 2 diabetes since these individuals would likely have insulin resistance.

## 5.7 References

1. World Health Organization. Obesity: preventing and managing the global epidemic: report of a WHO consultation. WHO Technical Report Series No. 894. 2000. Geneva, Switzerland: World Health Organization.
2. Wilson PW, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Archives of Internal Medicine* 2002;162:1867-1872.
3. Bray GA, Jablonski KA, Fujimoto WY, Barrett-Connor E, Haffner S, Hanson RL, Hill JO, Hubbard V, Kriska A, Stamm E, Pi-Sunyer FX. Relation of central adiposity and body mass index to the development of diabetes in the Diabetes Prevention Program. *American Journal of Clinical Nutrition* 2008;87:1212-1218.
4. Carey VJ, Walters EE, Colditz GA, Solomon CG, Willett WC, Rosner BA, Speizer FE, Manson JE. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. *American Journal of Epidemiology* 1997;145:614-619.
5. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 1994;17:961-969.
6. Wang Y, Rimm EB, Stampfer MJ, Willen WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. *American Journal of Clinical Nutrition* 2005;81:555-563.
7. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith Jr SC, Spertus JA, Costa F. Diagnosis and management of

- the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Executive summary. *Cardiology Reviews* 2005;13:322-327.
8. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005;365:1415-1428.
  9. Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, Balletshofer B, Machicao F, Fritsche A, Haring HU. Identification and characterization of metabolically benign obesity in humans. *Archives of Internal Medicine* 2008;168:1609-1616.
  10. Carey DG, Jenkins AB, Campbell LV, Freund J, Chisholm DF. Abdominal fat and insulin resistance in normal and overweight women: Direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes* 1996;45:633-638.
  11. Park KS, Rhee B, Lee KU, Kim S, Lee H, Koh C, Min H. Intra-abdominal fat is associated with decreased insulin sensitivity in healthy young men. *Metabolism* 1991;40:600-603.
  12. Balkau B, Deanfield JE, Despres JP, Bassand JP, Fox, KAA, Smith SC Barter P, Tan C, Van Gaal L, Wittchen HU, Massien C, Haffner SM. International day for the evaluation of abdominal obesity (IDEA): A study of waist circumference, cardiovascular disease, and diabetes mellitus in 168 000 primary care patients in 63 countries. *Circulation* 2007;116:1942-1951.
  13. McGarry JD, Dobbins RL. Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 1999;42:128-138.
  14. Shulman GI. Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy. *Physiology* 2004;19:183-190.



15. Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, Haring HU. Association of increased intramyocellular lipid content with insulin resistance in lean non-diabetic offspring of type 2 diabetic subjects. *Diabetes* 1999;48:1113-1119.
16. Krssak M, Petersen KF, Dresnar A, DiPietro L, Vogel SM, Rothman DL, Shulman GL, Roden M. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a  $^1\text{H}$  NMR spectroscopy study. *Diabetologia* 1999;42:113-116.
17. Frost G, Keogh B, Smith D, Akinsanya K, Leeds A. The effect of low-glycemic carbohydrate on insulin and glucose response in vivo and in vitro in patients with coronary heart disease. *Metabolism* 1996;45:669-672.
18. Wolever TMS, Bentum-Williams A, Jenkins DJA. Physiological modulation of plasma free fatty acid concentrations by diet: Metabolic implications in nondiabetic subjects. *Diabetes Care* 1995;18:962-970.
19. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unraveling the mechanism. *Lancet* 2010;375:2267-2277.
20. Goff LM, Frost GS, Hamilton G, Thomas EL, Dhillo WS, Dornhorst A, Bell JD. Carbohydrate-induced manipulation of insulin sensitivity independently of intramyocellular lipids. *British Journal of Nutrition* 2003;89:365-374.
21. National Cholesterol Education Program National Heart, Lung, and Blood Institute. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Executive Summary, National Institutes of Health. NIH Publication No. 01-3670, May 2001.

22. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St.Jeor S, Suttie J, Tribble DL Bazzarre TL. AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the nutrition committee of the American Heart Association. *Circulation* 2000;102:2284-2299.
23. The lipid research clinics population studies data book. Volume 2. The prevalence study-nutrient intake. Washington, D.C. Government Printing Office, National Institutes of Health. NIH Publication No. 82-2014, September 1982.
24. Baecke JAH, Burema J, Frijters, JER. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *American Journal of Clinical Nutrition* 1982;36:936-942.
25. World Health Organization. Physical Status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. Technical Report Series No 854, Annex 2. 1995;854:1-452.
26. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
27. Pacini G, Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. *Best Practice Research Clinical Endocrinology and Metabolism* 2003;17:305-322.
28. Saad MF, Anderson RL, Laws A, Watanabe RM, Kades WW, Chen YDI, Sands RE, Pei D, Savage PJ, Bergman RN. The Insulin Resistance Atherosclerosis Study: Comparison

between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. *Diabetes* 1994;43:1114-1121.

29. Friedewald WT, Levy RT, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. *Clinical Chemistry* 1972;18:499-502.
30. Bachmann OP, Dahl, DB, Brechtel K, Machann J, Haap M, Maier T, Lovischach M, Stumvoll M, Claussen CD, Schick F, Haring HU, Jacob S. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 2001;50:2579-2584.
31. Schrauwen-Hinderling VB, Kooi ME, Hesselink MKC, Moonen-Kornips E, Schaart G, Mustard KJ, Hardi G, Saris WHM, Nicolay K, Schrauwen P. Intramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet. *Obesity Research* 2005;13:2088-2094.
32. De Koning L, Gerstein HC, Bosch J, Diaz R, Mohan V, Dagenais G, Yusuf S, Anand SS. Anthropometric measures and glucose levels in a large multi-ethnic cohort of individuals at risk of developing type 2 diabetes. *Diabetologia* 2010;53:1322-1330.
33. Liese AD, Schulz M, Fang F, Wolever TMS, D'Agostino RB, Sparks KC, Mayer-Davis EJ. Dietary glycemic index and glycemic load, carbohydrate and fiber intake, and measures of insulin sensitivity, secretion, and adiposity in the insulin resistance atherosclerosis study. *Diabetes Care* 2005;28:2832-2838.
34. Ma Y, Olendzki B, Chiriboga, Hebert JR, Li Y, Li W, Campbell M, Gendreau K, Ockene IS. Association between dietary carbohydrates and body weight. *American Journal of Epidemiology* 2005;161:359-367.

35. Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, Smith SR, Alfonso A, Ravussin E. Effect of calorie restriction with or without exercise on insulin sensitivity,  $\beta$ -cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care* 2006;29:1337-1344.
36. Balkau B, Deanfield JE, Despres JP, Bassand JP, Fox KAA, Smith SC, Barter P, Tan CE, Van Gaal L, Wittchen HU, Massien C, Haffner SM. International day for the evaluation of abdominal obesity (IDEA). A study of waist circumference, cardiovascular disease, and diabetes mellitus in 168 000 primary care patients in 63 countries. *Circulation* 2007;116:1942-1951.
37. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Report of a WHO/IDF consultation. NLM Classification: WK 810, ISBN 9241594936, 2006. Geneva, Switzerland: World Health Organization [www.who.int/diabetes/publications/Definition%20and%20diagnosis%20of%20diabetes\\_new.pdf](http://www.who.int/diabetes/publications/Definition%20and%20diagnosis%20of%20diabetes_new.pdf)
38. Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 955,778 individuals followed for 2.4 years. *Diabetes Care* 1999;22:233-240.
39. Ford ES, Liu S. Glycemic index and serum high-density lipoprotein cholesterol concentration among US adults. *Archives of Internal Medicine* 2001;161:572-576.
40. Ludwig DS. The glycemic index. Physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *Journal of the American Medical Association* 2002;287:2414-2423.

41. Jenkins DJA, Kendall CWC, McKeown-Eyssen G, Josse RG, Silverberg J, Booth GL, Vidgen E, Josse AR, Nguyen TH, Corrigan S, Banach MS, Ares S, Mitchell S, Emam A, Augustin LSA, Parker TL, Leiter LA. Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes. *Journal of the American Medical Association* 2008;300:2742-2753.
42. Coulston AM, Reaven GM. Much ado about (almost) nothing. *Diabetes Care* 1997;20:241-243.
43. Jenkins DJA, Wolever TMS, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 1981;34:362-366.
44. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. *American Journal of Clinical Nutrition* 1991;54:846-854.
45. Wolever TM. Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values. *British Journal of Nutrition* 2004;91:295-300.

## **CHAPTER 6**

### **GLYCEMIC INDEX PREDICTS INDIVIDUAL GLUCOSE RESPONSES AFTER SELF- SELECTED BREAKFASTS IN FREE-LIVING, ABDOMINALLY OBESE ADULTS**

## **Glycemic Index Predicts Individual Glucose Responses after Self-Selected Breakfasts in Free-Living, Abdominally Obese Adults**

### **6.1 Introductory Statement**

Research has shown that the glycemic index (GI) predicts the postprandial glucose responses elicited by mixed meals in various populations. The degree to which an individual's glycemic response to a meal is determined by the GI and other components of the meal remains unclear, especially when meals are not consumed in a controlled setting. This study was conducted to determine whether GI is a significant determinant of individual glycemic responses elicited by self-selected breakfast meals in abdominally obese adults.

## 6.2 Abstract

**Background:** Research has shown that the glycemic index (GI) predicts the postprandial glucose responses elicited by mixed meals in various populations. The degree to which an individual's glycemic response to a meal is determined by the GI and other components of the meal remains unclear, especially when meals are not consumed in a controlled setting.

**Objective:** The purpose of this study was to test whether the GI of self-selected breakfast meals was a determinant of the individual glycemic responses in free-living adults with abdominal obesity.

**Design:** Free-living non-diabetic adults ( $n=57$ ) aged  $53.9\pm 9.8$  with a BMI of  $33.9\pm 5.3$  and waist-circumference (WC)  $109\pm 11$  cm underwent a 75g oral glucose tolerance test (OGTT) and, on a separate day, wore a continuous glucose monitoring system (CGMS) for 24h during which time they recorded all foods consumed. The protein, fat, available-carbohydrate (avCHO), and GI of the breakfast meals were calculated from the food records and the incremental areas under the glycemic response curves (iAUC) for 2h after breakfast ( $iAUC_{\text{breakfast}}$ ) were calculated from CGMS data. Values for  $iAUC_{\text{breakfast}}$ , avCHO, fat, fibre, and BMI were normalized by log-transformation. The ability of participant characteristics and breakfast composition to predict individual  $iAUC_{\text{breakfast}}$  response was determined using a step-wise multiple linear regression analysis.

**Results:** A total of 56% of the variation in  $iAUC_{\text{breakfast}}$  was explained by GI (30%,  $p<0.001$ ),  $iAUC$  after the OGTT (11%,  $p<0.001$ ), avCHO (11%,  $p<0.001$ ), and waist circumference (3%,  $p<0.05$ ). The effects fat, protein, dietary fibre, age, sex, and BMI were not significant ( $p>0.05$ ).

**Conclusions:** In free-living abdominally obese adults, GI is a significant determinant of individual glycemic responses elicited by self-selected breakfast meals.



### 6.3 Introduction

The glycemic index (GI) was developed in 1981 as a classification of the blood glucose-raising potential of carbohydrate containing foods (1) and is defined as the incremental area under the blood glucose response curve after consuming a 50 gram available-carbohydrate portion of a test food expressed as a percentage of the response after consuming 50 grams of oral anhydrous glucose by the same subject (2, 3). Research has demonstrated that low-GI diets improve glycemic control in diabetes (4, 5) and may decrease the risk of developing type 2 diabetes (6). Despite this evidence, there is controversy about the relevance of GI for free-living individuals because of concerns that it is difficult to choose low-GI foods, that GI values are imprecise, and that the GI does not predict the glycemic responses of individuals consuming normal mixed meals due to the high day-to-day variation of glycemic responses and the confounding effects of fat and protein (7, 8, 9, 10, 11). Previous studies showed that GI predicts the postprandial glucose responses elicited by mixed meals in groups of normal individuals (12, 13), adults with type 2 diabetes (14), and youths with type 1 diabetes (15). These studies examined the mean glycemic responses of groups of participants under controlled conditions. The question about whether the GI can predict individual glycemic responses to self-selected meals remains unclear. Variation in glycemic responses arises from at least four major sources: diurnal variation (time of day), meal-related factors, participant-related factors (between-individual variation), and unexplained day-to-day variation (within individuals) (16). The purpose of this study was to determine whether GI is a significant determinant of individual glycemic responses elicited by self-selected breakfast meals in abdominally obese adults. Only breakfast meals were included to remove the confounding effects of diurnal variation. Abdominally obese adults were studied because they represent a population at risk for

developing type 2 diabetes and may benefit from dietary approaches to reducing risk for diabetes.

## **6.4 Subjects and Methods**

This study was carried out on an outpatient basis at the Hamilton General Hospital, Centre for Cardiovascular Obesity Research and Management, McMaster University, Faculty of Health Sciences. The study protocol was approved by the Hamilton Health Sciences, McMaster University human research ethics board. All participants were given a participant information sheet and gave informed consent to participate in the study (Appendix 8.1). The trial is publicly registered with ClinicalTrials.gov, number NCT00147264.

### **6.4.1 Protocol**

This thesis chapter consists of results of an analysis of data collected at the end of the run-in period of a randomized 2x2 factorial design clinical trial which studied the effects of a low GI diet and telmisartan on intramyocellular lipids (TRIM trial (Telmisartan-Induced Reduction in Intra-Myocellular Lipids)).

### **6.4.2 Study Sample**

A total of 2433 participants from the general population responded through advertisement in local media and underwent a telephone screening process. Of the 171 participants who were invited for an initial screening visit to assess eligibility, 121 participants met inclusion criteria (refer to chapter 6 of this thesis) and were recruited for the main study. Participants were males and non-pregnant, non-lactating females aged 30-70yrs with abdominal obesity and a fasting plasma glucose <7.0 mmol/L. Abdominal obesity was defined as a waist circumference of >102 cm for males and >88 cm for females (17). Ninety-three of the 121 participants agreed to CGMS monitoring and of these, 57 met inclusion criteria for this study which included: food intake

including a breakfast meal, recorded for at least one day of the CGMS monitoring; valid and complete CGMS data on the day food intake was recorded; the time between breakfast and the next food intake being at least two hours; and a complete and valid OGTT. Reasons for exclusion were: 11 participants did not meet CGMS criteria, 9 did not record a food diary, 7 had an incomplete OGTT, one person did not eat breakfast, and 8 dropped out after the CGMS was inserted.

#### **6.4.3 Ad-Libitum Low Fat Diet**

After recruitment, the study participants underwent a 6 week dietary advice run in period during which time they followed a standardized low fat diet as outlined by the American Heart Association (19) consisting of 55% energy from carbohydrate, 30% from fat, less than 7% from saturated fat and 15% from protein. Daily energy requirements were estimated according to the Lipid Research Clinic Requirement formula (18) with an additional 300 kcal per day added on for exercise and daily energy expenditure (Appendix 8.2). Diets were prescribed on an ad-libitum basis. The aim of the diet was to be weight maintaining and to eliminate potential effects of variations in fat intake on IMCL content. Participants met with a nutrition counselor three times during the run in period and were given an information sheet (Appendix 8.3) to provide dietary advice on following a low fat diet. The participants completed three MEDFICTS dietary assessment questionnaires to assess dietary fat (17) and the Baecke habitual physical activity questionnaire was administered at the start-and-end of the study to determine activity levels (Appendix 8.6) (20). Participants were instructed to maintain their habitual level of physical activity throughout the study.

At the end of the run in period, participants completed a 3-day food diary (Appendix 8.5) starting on the second day of the CGMS monitoring. The composition of the breakfast meal

during one of the two days that included the CGMS monitoring was used for the purposes of this study. A valid breakfast meal was defined as: first food intake before noon of greater than 99 kcal, recorded for at least one day of the CGMS monitoring; and, the time between breakfast and the next food intake being at least two hours (because GI is a measure of glycemic response over two hours). If valid data existed for more than one breakfast meal, one meal per participant was chosen at random for analysis.

Macronutrients and GI of test foods were calculated using the Food Processor SQL Nutrition Analysis & Fitness software package version 9.5 (ESHA Research, Salem, OR, USA) for GI and macronutrient composition as a measure of the participant's habitual dietary intake. The GI values for the foods were expressed with the GI of glucose = 100 and derived from published tables by using locally tested values where possible, as previously described by Wolever et al (13). The GI of the breakfast meal was calculated as the sum of, for all foods in the breakfast meal, the amount of available carbohydrate (avCHO) in the portion of food consumed ( $g_f$ ) multiplied by (the GI of that food ( $GI_f$ ) divided by the amount of avCHO in the breakfast meal) ( $g_b$ )), ( $g_f \times GI_f / g_b$ ) (13).

#### **6.4.4 Continuous Glucose Monitoring System**

One to two weeks before the end of the run in period, participants underwent 24 hour continuous glucose monitoring for three days using a Medtronic MiniMed CGMS monitor (Medtronic-MiniMed, Northridge, CA, USA). The CGMS monitor included a disposable glucose indwelling sensor with an external electrical connector that was inserted using a sensor inserter applicator into the subcutaneous tissue of the abdominal wall to measure interstitial fluid, a glucose monitor that recorded a mean of 30 signals from the sensor every 5 minutes for a total of 288 readings for a 24 hour period, and a communication device (Com-Station) enabling data

stored in the monitor to be downloaded into a computer (21). The participants were instructed on the use of the CGMS and a blood glucose meter (Ascensia™ Contour™, Bayer HealthCare LLC, Mishawaka, IN, USA) to enter four blood glucose values per day to calibrate the CGMS monitor. A valid and complete CGMS data on the day food intake was recorded was defined as 288 sensor readings, at least three meter readings entered by the participant in the blood glucose meter, and no sensor errors detected. The start of breakfast for the CGMS data analysis was determined by examining the CGMS glucose values near the time the participant indicated that breakfast was consumed to find when blood glucose started to increase, defined as when the second of two successive glucose readings differed by  $\geq 0.2$  mmol/L from the first. Fasting blood glucose was taken to be the mean of the four values (0, 5, 10, and 15 minutes) before the first increase in blood glucose. The glycemic response was measured for 120 minutes (24 readings) after the start of breakfast ( $iAUC_{\text{breakfast}}$ ).

#### **6.4.5 Oral Glucose Tolerance Test**

At the end of the run in period, participants underwent a 75 gram oral glucose tolerance test (OGTT) after a 12 hour fast. An indwelling catheter was inserted in the forearm and venous blood samples were obtained for plasma glucose at 15, 10, and 5 minutes prior to ingestion of the glucose load (75 gram solution of dextrose) and then again at 30, 60, and 120 minutes after starting the glucose drink. Fasting plasma glucose was taken to be the mean of the three readings following the fast. Glucose was measured by a glucose oxidase method and serum insulin was measured with an immunometric assay (Diagnostic Products Corporation, Los Angeles, CA). A complete and valid OGTT was defined as obtaining blood samples at 0, 30, 60 and 120 minutes ( $iAUC_{\text{OGTT}}$ ).

#### 6.4.6 Anthropometric Measurements

Height (cm) was measured at the initial screening visit to the nearest 0.1 cm using a wall mounted stadiometer and body weight was measured at every visit to the nearest 0.1 kg on a digital weigh scale. Body mass index (BMI) was calculated as body weight (kg) divided by height ( $m^2$ ). Waist circumference (WC) was measured to the nearest 0.1 cm at the beginning at end of the run in period, using the World Health Organization (WHO) method (mid-point between the palpated inferior border of the last rib and upper border of the iliac crest in a horizontal plane at the end of normal expiration) (22).

#### 6.4.7 Statistical Analysis

All data are presented as means  $\pm$ SD unless otherwise indicated. Calculations for  $iAUC_{\text{breakfast}}$  and  $iAUC_{\text{OGTT}}$ , ignoring area beneath the baseline were determined as previously described (23). The values for  $iAUC_{\text{breakfast}}$  were divided into tertiles, and the mean values for  $iAUC_{\text{OGTT}}$ , protein, fat, avCHO, dietary fibre, and GI for participants within the  $iAUC_{\text{breakfast}}$  tertiles were compared by ANOVA. The independent contributions of  $iAUC_{\text{OGTT}}$ , BMI, WC, protein, fat, avCHO, dietary fibre, and GI to predicting  $iAUC_{\text{breakfast}}$  was determined by step-wise multiple linear regression (Lotus 123, Lotus Development) using the step-up procedure (24), with age and sex included in all models (because some anthropometric and breakfast intake variables were significantly related to age and sex). The variable with the most significant correlation with  $iAUC_{\text{breakfast}}$  was added to the model first, all remaining variables were then tested, and the most significant added sequentially to the model until no further significant reduction in the residual variation was obtained. Prior to regression analysis, non-normally distributed variables based on D'Agostino's test ( $iAUC_{\text{breakfast}}$ , avCHO, BMI, fat, and fibre) were normalized by log-transformation. Statistical significance was taken to be 2-tailed  $p < 0.05$ , with

comparisons between individual means adjusted for multiple comparisons using Tukey's method.

## 6.5 Results

### 6.5.1 Subject Characteristics

Entering the study, participants mean age was  $53.9 \pm 9.8$  years and 68% were female. All participants had abdominal obesity ( $109 \pm 11$  cm) (17) with a BMI of  $33.9 \pm 5.3$  (22). Age, BMI, and WC were significantly correlated with total dietary fat (g) ( $r=0.28$ ,  $p<0.05$ ;  $r=0.27$ ,  $p<0.05$ ;  $r=0.27$ ,  $p<0.05$ , respectively). WC was also correlated with BMI ( $r=0.68$ ,  $p<0.001$ ) and avCHO (g) ( $r=0.37$ ,  $p<0.01$ ). Clinical characteristics of the study population are listed in Table 6.1.

**Table 6.1.** Clinical Characteristics of the Study Population Divided by Tertile of  $iAUC_{\text{breakfast}}$ .

	All Participants	Tertile 1	Tertile 2	Tertile 3
Variable	N = 57	N = 19	N = 19	N = 19
Sex (M:F), N	18:39	6:13	6:13	6:13
Age (years)	$53.9 \pm 9.8$ (37-69)	$54.8 \pm 8.9$	$52.4 \pm 11.8$	$54.3 \pm 9.8$
BMI (kg/m <sup>2</sup> )	$33.9 \pm 5.3$ (27-39)	$32.3 \pm 2.9$	$36.1 \pm 5.8$	$33.1 \pm 6.1$
Waist circumference (cm)	$109 \pm 11$ (93-122)	$108 \pm 8$	$113 \pm 13$	$106 \pm 11$

Data are means  $\pm$  SD; brackets ( ) are range; M, Males; F, Females

### 6.5.2 OGTT, CGMS and Dietary Intake

The OGTT showed 36 participants with normal fasting glucose ( $<5.6$  mmol/L) of whom 19 had normal plasma glucose two hours after 75 grams oral glucose (2hPCG) ( $<7.8$  mmol/L), 16 had impaired glucose tolerance (IGT) ( $2hPCG \geq 7.8$  to  $<11.1$  mmol/L), and one had diabetes

(2hPCG  $\geq 11.1$  mmol/L); 20 participants had impaired fasting glucose (IFG) (fasting glucose of  $\geq 5.6$  to  $< 7.0$  mmol/L) of whom 8 had normal 2hPCG, 6 had IGT, and 6 had a diabetic 2hPCG; one individual had diabetic values for both fasting and 2hPCG. Participants with diabetes based on the OGTT had higher  $iAUC_{OGTT}$  ( $405 \pm 41$  vs  $232 \pm 74$  mmol/L x min/L) and  $iAUC_{breakfast}$  ( $204 \pm 92$  vs  $114 \pm 83$  mmol x min/L) compared to the participants without diabetes. The GI and avCHO intake at breakfast did not significantly differ between the participants with and without diabetes ( $p > 0.05$ ) (GI,  $64 \pm 10$  vs  $60 \pm 9$ g; avCHO,  $60 \pm 21$  vs  $68 \pm 40$ g, respectively).

Mean fasting glucose before breakfast was similar across the tertiles of  $iAUC_{breakfast}$  (Table 6.2). Participants in the highest tertile of  $iAUC_{breakfast}$  had significantly higher glycemic responses after breakfast (Figure 6.1), higher fasting glucose before the OGTT, and a higher  $iAUC_{OGTT}$  than those in the lowest tertile of  $iAUC_{breakfast}$  ( $p < 0.05$ ) (Table 6.2). Despite large ranges of intakes of avCHO, fat, protein, and fibre, only GI significantly differed between the highest and lowest tertiles of  $iAUC_{breakfast}$  ( $p < 0.05$ ) (Table 6.2).

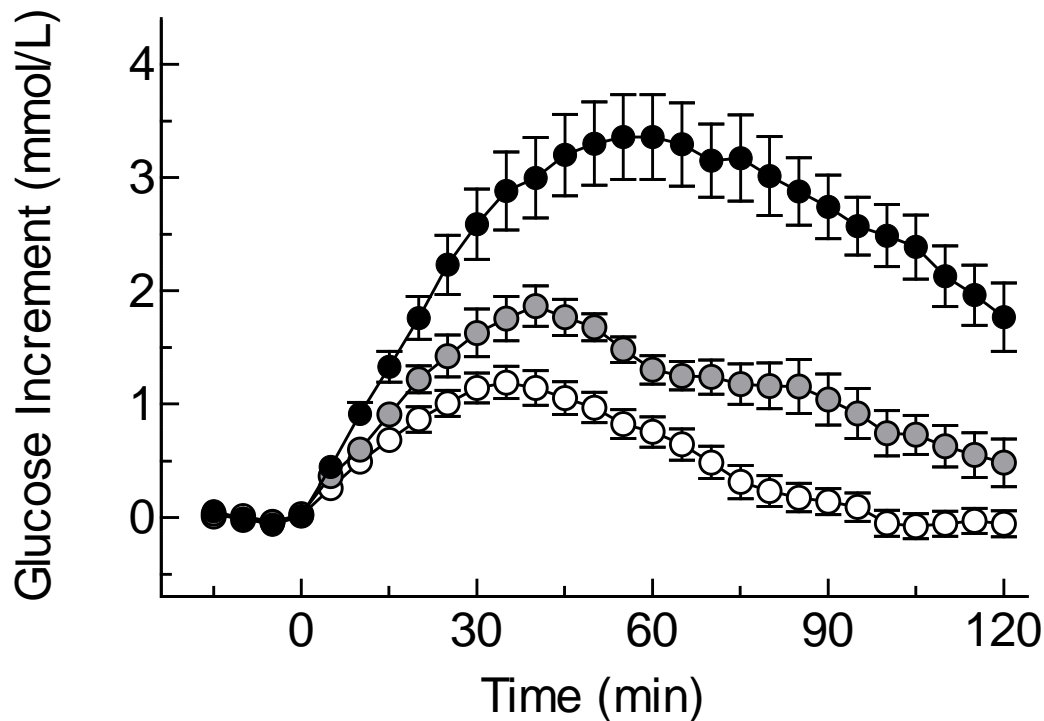
$iAUC_{breakfast}$  was significantly correlated with GI ( $r = 0.55$ ,  $p < 0.001$ ), avCHO ( $r = 0.35$ ,  $p < 0.01$ ), OGTT 2hPCG ( $r = 0.40$ ,  $p < 0.01$ ), and  $iAUC_{OGTT}$  ( $r = 0.38$ ,  $p < 0.01$ ). Neither  $iAUC_{OGTT}$  nor  $iAUC_{breakfast}$  were significantly related to age, sex, BMI, WC, protein, fat, or fibre, and no significant relationship was found between GI and avCHO ( $p > 0.05$ ).

When considered individually, the variable that explained most of the variation in  $iAUC_{breakfast}$  was GI ( $r^2 = 0.30$ ) followed by 2hPCG ( $r^2 = 0.16$ ),  $iAUC_{OGTT}$  ( $r^2 = 0.14$ ), and avCHO ( $r^2 = 0.12$ ). Multiple regression analysis showed that, whereas age and sex were not significantly related to  $iAUC_{breakfast}$ , GI, avCHO and  $iAUC_{OGTT}$  had significant independent effects that together explained 56% of the variation in  $iAUC_{breakfast}$  (GI, standardized  $\beta = 0.47 \pm 0.09$ ,  $p < 0.001$ ; avCHO, standardized  $\beta = 0.43 \pm 0.11$ ,  $p < 0.001$ ;  $iAUC_{OGTT}$ , standardized  $\beta = 0.36 \pm 0.09$ ,



$p < 0.001$ ). WC, BMI, protein, fat, and fibre had no significant effects when added to the model ( $p > 0.05$ ).

When the 8 participants with diabetes by OGTT or fasting glucose were excluded, the results of the multiple regression analysis were similar to those for the total population, with age (standardized  $\beta = -0.15 \pm 0.11$ ,  $p = 0.17$ ), sex (standardized  $\beta = 0.16 \pm 0.14$ ,  $p = 0.24$ ), GI (standardized  $\beta = 0.51 \pm 0.11$ ,  $p < 0.001$ ), avCHO (standardized  $\beta = 0.45 \pm 0.13$ ,  $p < 0.001$ ), and  $iAUC_{OGTT}$  (standardized  $\beta = 0.28 \pm 0.12$ ,  $p < 0.05$ ) explaining 51% of the variation in  $iAUC_{breakfast}$ .



**Figure 6.1.**  $iAUC_{breakfast}$  for 57 free-living, abdominally obese adults by tertile of  $iAUC_{breakfast}$ .

Values are means  $\pm$  SEM,  $n = 19$  per tertile group. Tertile 1 (○), Tertile 2 (●), Tertile 3 (●).

$iAUC_{breakfast}$ , incremental area under the glycemic response curve for 2 hours after breakfast.

**Table 6.2.** CGMS, OGTT and Breakfast Meal Composition of Abdominally Obese AdultsDivided by Tertile of iAUC<sub>breakfast</sub>.

	All Participants	Tertile 1	Tertile 2	Tertile 3
Variable	N = 57	N = 19	N = 19	N = 19
iAUC <sub>breakfast</sub> (mmol×min/L)	127±89 (16-456)	52.9±16.5 <sup>a</sup> (16-81)	104.6±15.6 <sup>b</sup> (84-132)	222.5±91.6 <sup>c</sup> (133-456)
iAUC <sub>OGTT</sub> (mmol×min/L)	256±93 (65-478)	214±85 <sup>a</sup>	264±77 <sup>ab</sup>	292±102 <sup>b</sup>
Breakfast fasting glucose (mmol/L)	6.0±1.0 (3.2-8.9)	5.9±1.0	5.8±1.1	6.2±1.0
OGTT fasting glucose (mmol/L)	5.5±0.6 (4.4-7.6)	5.3±0.5 <sup>a</sup>	5.5±0.8 <sup>ab</sup>	5.7±0.6 <sup>b</sup>
OGTT FG status (N:IFG:D)	36:20:1	15:4:0	12:6:1	9:10:0
OGTT 2hPCG status (N:IFG:D)	27:22:8	13:6:0	8:9:2	6:7:6
<b>Composition of Breakfast Meal</b>				
Energy (kcal)	400±187 (124-1136)	350±112	403±156	447±259
Protein (g)	17±8 (3-43)	16±7	17±8	16±10
Fat (g)	7±5 (1-28)	8±5	7±7	8±4
Available carbohydrate (g)	67±38 (16-222)	55±24	67±28	79±53
Glycemic Index (%)	60±9 (37-85)	54±8 <sup>a</sup>	62±8 <sup>b</sup>	65±8 <sup>b</sup>

Data are means ± SD; brackets ( ) are range; means in a row with superscripts without a common letter<sup>ab</sup> differ, p<0.05. iAUC<sub>breakfast</sub>, incremental area under the glycemic response curve for 2 hours after breakfast; iAUC<sub>OGTT</sub>, incremental area under the glycemic response curve for 2 hours after 75 grams oral glucose; OGTT, oral glucose tolerance test; N, normal; IGT, impaired glucose tolerance; D, diabetic; IFG, impaired fasting glucose; FG, fasting glucose; 2hPCG, plasma glucose 2 hours after 75 grams oral glucose.

## 6.6 Discussion and Conclusions

The results showed that in free-living participants with nondiabetic fasting glucose on recruitment, a high WC, and a wide range of nutrient intakes, the GI of self-selected breakfast meals varied over a considerable range and was a highly significant determinant of individual glycemic responses. The 2.3-fold variation in meal GI (37-85) was a more important determinant of  $iAUC_{\text{breakfast}}$  than the 13.8-fold variation in recorded avCHO intake (16-222 g). The variation in recorded protein (3-43 g), fat (1-28 g), and fibre (0-56 g) intakes had a negligible effect.

The present results are consistent with those of previous studies (13, 25) showing that GI was a significant determinant of the glycemic response elicited by mixed breakfast meals containing variable amounts of energy, avCHO, protein, fat, and fibre. In the present study however, avCHO and GI together explained approximately 40% of the variation in  $iAUC$  compared to approximately 90% in previous studies. There are two main reasons for this; one is because each value of  $iAUC$  used in the regression analysis was the response of a single individual on one occasion as opposed to the mean value for 8 to 12 individuals in previous studies. The second reason is that in earlier studies, between-individual variation was reduced to zero by having every participant test all of the test meals. In this study, every participant ate a different test meal therefore the variation in  $iAUC_{\text{breakfast}}$  includes between-individual variation. Further, this study used  $iAUC_{\text{OGTT}}$  to control for between-individual variation, but  $iAUC_{\text{OGTT}}$  is an imprecise estimate of each person's true response because of within-individual variation.

Upon enrollment, all participants met the eligibility criterion of a nondiabetic fasting glucose of  $<7.0$  mmol/L, however, during the OGTT, 8 participants had 2hPCG values in the diabetic range of  $\geq 11.1$  mmol/L and one of these participants had a fasting glucose in the

diabetic range of 7.6 mmol/L. The differences in classification based on 2hPCG compared to fasting glucose were not unexpected because raised 2hPCG tends to occur earlier in the natural history of type 2 diabetes than raised fasting glucose (26). Further, studies have shown that when individuals not known to have diabetes are screened with an OGTT, 30-40% of those with a 2hPCG in the diabetic range have nondiabetic fasting glucose (27, 28, 29). Day-to-day variations in fasting glucose, occurring presumably due to variations in recent diet, activity, sleep, stress, and illness, may account for differences in classification of diabetes on repeated testing. In this study, participants who had diabetic 2hPCG were not excluded from the primary analysis because they met inclusion criteria of a normal fasting glucose on screening. Although the results with respect to the ability of GI to predict glycemic responses were not changed by excluding participants with diabetic 2hPCG, the effect of  $iAUC_{OGTT}$  was reduced because of the reduced range of  $iAUC_{OGTT}$  values after excluding those with diabetic 2hPCG values.

The results of this study showed that variation in the protein and fat content of self-selected breakfast meals had a negligible effect on the glycemic responses they elicited. It is generally considered that protein and fat reduce glycemic responses by delaying gastric emptying and increasing insulin secretion (8, 30). The interquartile ranges (25<sup>th</sup> to 75<sup>th</sup> percentiles) for our participants' protein and fat intakes were 10-20g and 3-10g respectively, and the 10<sup>th</sup> and 90<sup>th</sup> percentiles were 8-27g and 2-13g, which was within the range used in previous studies examining whether adding 10-20g protein or 5-15g fat to avCHO reduces glycemic responses (31, 32, 33, 34, 35). The results of these previous studies however are inconsistent; the effect of 10-20 g protein varies from 0 (31, 32) to modest (15% to 40%) (33, 34), to large (40% to 50%) (35), and the effect of 5-15 g fat varied across a similar range (33, 34, 36, 37, 38, 39). The results of this study do not challenge the concept that adding fat and protein to avCHO reduces

glycemic responses but rather challenge the ability to extrapolate the results of this experimental design to normal mixed meals. The experimental model of adding protein or fat to a fixed amount of avCHO does not reflect normal eating patterns in which meals vary in the amounts of all the nutrients they contain. To maintain energy balance, meals high in protein or fat would be low in avCHO which would have more influence on the glycemic response. For example, adding 15g fat to 50g avCHO from bread would reduce the glycemic response by approximately 20% (38), however, to make a meal isocaloric, the amount of avCHO would have to be reduced to 16g, which would reduce the glycemic response by approximately 55% (40).

A perceived barrier to the clinical use of GI is a concern that it limits food choice (41), however many commonly eaten foods have a low GI. In this study, 28% of self-selected breakfast meals had a low GI (i.e.  $\leq 55$ ), thus the barrier may not be that the GI limits food choice because they are uncommon but rather because it is difficult to know which specific foods have a low GI. This difficulty arises because most foods are not labeled with their GI value, and the GI values of foods reported in the International GI Tables (42) vary considerably. For example, there are 100 GI values for various types of rice (42), which 34% are low GI ( $< 56$ ) and 33% are high GI ( $> 69$ ). The present results do not address this issue directly, although they show it is possible to select GI values for the foods recorded on a food record that predict the glycemic response elicited by a mixed meal. It has been suggested that the variation in GI values for similar foods is due to imprecise method of measuring GI (10), however when performed correctly, the GI method is precise enough to distinguish between low GI and high GI foods with 95% certainty (43). This suggests that the variation of GI values for similar foods arises either from use of incorrect methods or from real differences among foods due to differences in starch

structure (44, 45, 46) related to genetic variety (47, 48), food processing, cooking, storage, and serving methods (49, 50, 51).

A limitation of this study was that only the glycemic response elicited by breakfast was considered, therefore the present results cannot necessarily be extrapolated to other meals of the day. The glycemic response after lunch and dinner depends on many factors other than the composition of the meal. These include the composition of the previous meal, the time interval between meals, and the time of day (52, 53, 54, 55, 56, 57, 58). Another limitation of this study is that only 57 of the 121 participants (47%) from the primary study were included. This number is small in relation to the number of variables available for inclusion into the multiple regression model, thus the results need to be interpreted with caution. In addition, the participants in this study were all abdominally obese. The relative importance of the variables studied here in determining glycemic responses may vary in different populations.

In conclusion, GI was a significant determinant of individual glycemic responses elicited by self-selected breakfast meals in free-living, abdominally obese adults.

## 6.7 References

1. Jenkins DJA, Wolever TMS, Taylor RH, Barker HM, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American Journal of Clinical Nutrition* 1981;34:362-366.
2. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. *American Journal of Clinical Nutrition* 1991;54:846-854.
3. Wolever TM. Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values. *British Journal of Nutrition* 2004;91:295-300.
4. Brand-Miller J, Hayne S, Petocz P, Colagiuri S. Low-glycemic index diets in the management of diabetes. *Diabetes Care* 2003;26:2261-2267.
5. Jenkins DJA, Kendall CWC, McKeown-Eyssen G, Josse RG, Silverberg J, Booth GL, Vidgen E, Josse AR, Nguyen TH, Corrigan S, Banach MS, Ares S, Mitchell S, Emam A, Augustin LSA, Parker TL, Leiter LA. Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes. *Journal of the American Medical Association* 2008;300:2742-2753.
6. Barclay AW, Petocz P, McMillan-Price J *et al.* (2008) Glycemic load and chronic disease risk-a meta-analysis of observational studies. *American Journal of Clinical Nutrition* 2008;87:627-637.
7. Williams SM, Venn BJ, Perry T *et al.* (2008) Another approach to estimating the reliability of glycaemic index. *British Journal of Nutrition* **100**, 364-372.
8. Pi-Suyet FX. Glycemic index and disease. *American Journal of Clinical Nutrition* 2002;76:290S-2908S.

9. Franz MJ. The Glycemic Index: Not the most effective nutrition therapy intervention. *Diabetes Care* 2003;26:2466-2468.
10. DeVries JW. (2007) The glycemic index: the analytical perspective. *Cereal Foods World* 2007;52:45-49.
11. Vega-López S, Ausman LM, Griffith JL *et al.* (2007) Interindividual variability and intra-individual reproducibility of glycemic index values for commercial white bread. *Diabetes Care* 2007;30:1412-1417.
12. Brynes AE, Adamson J, Dornhorst A, Frost GS. The beneficial effect of a diet with low glycaemic index on 24 h glucose profiles in healthy young people as assessed by continuous glucose monitoring. *British Journal of Nutrition* 2005;93:179-182.
13. Wolever TMS, Yang M, Yi Zeng X, Atkinson F, Brand-Miller JC. Food glycemic index, as given in Glycemic Index tables, is a significant determinant of glycemic responses elicited by composite breakfast meals. *American Journal of Clinical Nutrition* 2006;83:1306-1312.
14. Brynes AE, Lee JL, Brighton RE, Leeds AR, Dornhorst A, Frost GS. A low glycemic diet significantly improves the 24-h blood glucose profile in people with type 2 diabetes, as assessed using the continuous glucose MiniMed monitor. *Diabetes Care* 2003;26:548-549.
15. Nansel TR, Gellar L & McGill A. Effect of varying glycemic index meals on blood glucose control assessed with continuous glucose monitoring in youth with type 1 diabetes on basal-bolus insulin regimens. *Diabetes Care* 2008;31:695-697.
16. Franc S, Dardari D, Peschard C, Riveline JP, Biedzinski M, Boucherie B, Petit C, Requeda E, Mistretta F, Varroud-Vial M, Charpentier G. Can postprandial blood glucose excursion be predicted in type 2 diabetes? *Diabetes Care* 2010;33:1913-1918.



17. National Cholesterol Education Program National Heart, Lung, and Blood Institute. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Executive Summary, National Institutes of Health. NIH Publication No. 01-3670, May 2001.
18. The lipid research clinics population studies data book. Volume 2. The prevalence study-nutrient intake. Washington, D.C. Government Printing Office, National Institutes of Health. NIH Publication No. 82-2014, September 1982.
19. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St.Jeor S, Suttie J, Tribble DL Bazzarre TL. AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the nutrition committee of the American Heart Association. *Circulation* 2000;102:2284-2299.
20. Baecke JAH, Burema J, Frijters, JER. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *American Journal of Clinical Nutrition* 1982;36:936-942.
21. Mastrototaro JJ. The MiniMed Continuous Glucose Monitoring System. *Diabetes Technology and Therapeutics* 2000;2:S13-S18.
22. World Health Organization. Physical Status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. Technical Report Series No 854, Annex 2. 1995;854:1-452.
23. Wolever TM, Jenkins DF, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. *American Journal of Clinical Nutrition* 1991;54:846-854.

24. Snedecor GW, Cochran WG. Statistical methods. 7<sup>th</sup> edition. Ames (IA): Iowa State University Press; 1980.
25. Wolever TMS, Bolognesi C. Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index. *Journal of Nutrition* 1996;126:2807-2812.
26. DeFronzo RA. The triumvirate:  $\beta$ -cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 1988;37:667-687.
27. Shaw JE, Zimmet PZ, de Courten M, Dowse GK, Ghitson P, Gareeboo H, Hemraj F, Fareed D, Tuomilehto J, Alberti KGMM. Impaired fasting glucose or impaired glucose tolerance: what best predicts future diabetes in Mauritius? *Diabetes Care* 1999;22:399-402.
28. Kousta E, Lawrence NJ, Penn A, Millauer BA, Robinson S, Dornhorst A, de Swiet M, Steer PJ, Grenfeli A, Mather HM, Johnston DG, McCarthy MI. Implications of new diagnostic criteria for abnormal glucose homeostasis in women with previous gestational diabetes. *Diabetes Care* 1999;22:933-937.
29. Wolever TMS, Mehling C. Screening for diabetes and impaired glucose tolerance (IGT): lack of sensitivity of fasting plasma glucose. *Canadian Journal of Diabetes Care* 1999;23:23-28.
30. Hollenbeck CB, Coulston AM. The clinical utility of the glycemic index and its application to mixed meals. *Canadian Journal of Physiology and Pharmacology* 1991;69:100-107.
31. Westphal SA, Gannon MC, Nuttall FQ. Metabolic response to glucose ingested with various amounts of protein. *American Journal of Clinical Nutrition* 1990;52:267-272.
32. Liljeberg Elmstahl H, Bjorck I. Milk as a supplement to mixed meals may elevate postprandial insulinaemia. *European Journal of Clinical Nutrition* 2001;55:994-999.

33. Moghaddam E, Vogt JA, Wolever TMS. The effects of fat and protein on glycemic responses in non-diabetic humans vary with waist circumference, fasting plasma insulin and dietary fiber intake. *Journal of Nutrition* 2006;136:2506-2511.
34. Lan-Pidhainy X, Wolever TMS. The hypoglycemic effect of fat and protein is not attenuated by insulin resistance. *American Journal of Clinical Nutrition* 2010;91:98-105.
35. Spiller GA, Jensen CD, Pattison TS, Chuck CS, Whittam JH, Scala J. Effect of protein dose on serum glucose and insulin response to sugars. *American Journal of Clinical Nutrition* 1987;46:474-480.
36. Simpson RW, McDonald J, Wahlqvist ML, Atley L, Outch K. Macronutrients have different metabolic effects in nondiabetics and diabetics. *American Journal of Clinical Nutrition* 1985;42:449-453.
37. Murphy MC, Isherwood SG, Sethi S, Gould BJ, Wright JW, Knapper JA, Williams CM. Postprandial lipid and hormone responses to meals of varying fat contents: modulatory role of lipoprotein lipase? *European Journal of Clinical Nutrition* 1995;578-588.
38. Owen B, Wolever TMS. Effect of fat on glycaemic responses in normal subjects: a dose-response study. *Nutrition Research* 2003;23:1341-1347.
39. Normand S, Khalfallah Y, Louche-Pelissier C, Pachiaudi C, Antoine J, Blanc S, Desage M, Riou J, Laville M. Influence of dietary fat on postprandial glucose metabolism (exogenous and endogenous) using intrinsically <sup>13</sup>C-enriched durum wheat. *British Journal of Nutrition* 2001;86:3-11.
40. Wolever TMS, Gibbs AL, Spolar M, Hitchner EV, Heimowitz C. Equivalent glycemic load (EGL): a method for quantifying the glycemic responses elicited by low carbohydrate foods. *Nutrition and Metabolism* 2006;3:33.

41. Brekke HK, Sunesson A, Axelsen M, Lenner RA. Attitudes and barriers to dietary advice aimed at reducing risk of type 2 diabetes in first-degree relatives of patients with type 2 diabetes. *Journal of Human Nutrition and Dietetics* 2004;17:513-521.
42. Atkinson FS, Foster-Powell K, Brand-Miller J. International tables of glycemic index and glycemic load values: 2008. *Diabetes Care* 2008;31:2281-2283.
43. Wolever TMS, Brand-Miller JC, Abernethy J, Astrup A, Atkinson F, Axelsen M, Bjorck I, Brighenti F, Brown R, Brynes A, Casiraghi MC, Cazaubiel M, Dahlqvist L, Delport e, Denyer GS, Erba D, Frost G, Granfeldt Y, Hampton S, Hart VA, Hatonen KA, Henry CJ, Hertzler S, Hull S, Jerling J, Johnston KL, Lightowler H, Mann N, Morgan L, Panlasigui LN, Pelkman C, Perry T, Pfeiffer AFH, Pieters m, Ramdath DD, Ramsingh RT, Robert SD, Robinson C, Sarkkinen E, Scazzina F, Sison DCD, Sloth B, Staniforth J, Tapola N, Valsta LM, Verkooijen I, Weickert MO, Weseler AR, Wilkie P, Zhang J. Measuring the glycemic index of foods: interlaboratory study. *American Journal of Clinical Nutrition* 2008;87(suppl):247S-257S.
44. Granfeldt Y, Drews A, Bjorck I. Arepas made from high amylose corn flour produce favorably low glucose and insulin responses in healthy humans. *Journal of Nutrition* 1995;125:459-465.
45. Hoebler C, Karinthi A, Chiron H, Champ M, Barry JL. Bioavailability of starch in bread rich in amylose; metabolic responses in healthy subjects and starch structure. *European Journal of Clinical Nutrition* 1999;53:360-366.
46. Zhang G, Hamaker BR. Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. *Critical Reviews in Food Science and Nutrition* 2009;49:852-867.

47. Tian Z, Qian Q, Liu , Yan M, Liu X, Yan C, Liu G, Gao Z, Tang S, Zeng D, Wang Y, Yu J, Gu M, Li J. Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. *Proceedings of the National Academy of Science* 2009;106:21760-21765.
48. Gray D, Abdel-Aal E-SM, Seetharaman K, Kakuda Y. Differences in carbohydrate composition and digestion in vitro of selected barley cultivars as influenced by pearling and cooking. *Cereal Chemistry Journal* 2009;86:669-678.
49. Granfeldt Y, Hagander B, Bjorck I. Metabolic responses to starch in oat and wheat products. On the importance of food structure, incomplete gelatinization or presence of viscous dietary fibre. *European Journal of Clinical Nutrition* 1995;49:189-199.
50. Akerberg A, Liljeberg H, Bjorck I. Effects of amylose/amylopectin ratio and baking conditions on resistant starch formation and glycaemic indices. *Journal of Cereal Science* 1998;28:71-80.
51. Fernandes G, Velangi A, Wolever TMS. Glycemic index of potatoes commonly consumed in North America. *Journal of the American Dietetic Association* 2005;105:557-562.
52. Jenkins DJA, Wolever TMS, Taylor RH, Griffiths C, Krzeminska K, Lawrie JA, Bennett CM, Goff DV, Sarson DL, Bloom SR. Slow release carbohydrate improves second meal tolerance. *American Journal of Clinical Nutrition* 1982;35:1339-1346.
53. Collier GR, Wolever TMS, Jenkins DJA. Concurrent ingestion of fat and reduction in starch content impairs carbohydrate tolerance to subsequent meals. *American Journal of Clinical Nutrition* 1987;45:963-969.

54. Nestler JE, Barlaschini CO, Clore JN, Blackard WG. Absorption characteristic of breakfast determines insulin sensitivity and carbohydrate tolerance for lunch. *Diabetes Care* 1988;11:755-760.
55. Wolever TMS, Bentum-Williams A, Jenkins DJA. Physiologic modulation of plasma FFA concentrations by diet: metabolic implications in non-diabetic subjects. *Diabetes Care* 1995;18:962-970.
56. Liljeberg HGM, Akerberg AKE, Bjorck IME. Effect of the glycemic index and content of indigestible carbohydrates of cereal-based breakfast meals on glucose tolerance at lunch in healthy subjects. *American Journal of Clinical Nutrition* 1999;647-655.
57. Malherbe C, de Gasparo M, de Hertogh R, Hoet JJ. Circadian variations of blood sugar and plasma insulin levels in man. *Diabetologia* 1969;5:397-404.
58. Service FJ, Hall LD, Westland RE, O'Brien PC, Go VLW, Haymond MW, Rizza RA. Effects of size, time of day and sequence of meal ingestion on carbohydrate tolerance in normal subjects. *Diabetologia* 1983;25:316-321.

**CHAPTER 7**  
**GENERAL DISCUSSION AND CONCLUSIONS**

## 7.1 General Discussion

Obesity has been on the rise in recent years and is now considered to be a worldwide epidemic (1). Abdominal obesity, characterized by the accumulation of visceral fat, is now recognized as an independent risk factor for cardiovascular disease and type 2 diabetes (2). Abdominally obese individuals also have elevated stores of fat in skeletal muscle, specifically intramyocellular lipids (IMCL), which appear to reduce insulin-stimulated glucose uptake and cause insulin resistance (3, 4). Strategies for reducing risk factors for diseases in these individuals include improving insulin sensitivity (5).

Studies examining the role of dietary fat and insulin sensitivity found that IMCL increased with high-fat feeding and insulin sensitivity decreased (6, 7). The primary purpose of the first study (run-in phase) in this thesis was to eliminate potential effects of variations in dietary fat intake which may influence IMCL content in the main study. Participants in the run-in phase significantly decreased dietary total fat ( $p<0.0001$ ) and saturated fat ( $p<0.01$ ) from advice on following a low-fat diet and there was a significant relationship between IMCL and dietary fat ( $p<0.05$ ) but not with insulin sensitivity. A limitation of the run-in study was that IMCL content was measured only at the end of the study, therefore conclusions cannot be made on whether changes occurred in IMCL and if there was any effect on insulin sensitivity after reducing fat in the diet. On the other hand, it was also hypothesized that following dietary advice on lowering overall fat and saturated fat intake would improve metabolic profiles in adults with abdominal obesity. The blood cholesterol data supported this hypothesis with an approximate 9% decrease in total and LDL cholesterol. This is an important finding given that participants followed a short-term (4 to 6 weeks), ad-libitum, weight-maintaining, low-fat dietary advice study. Since abdominally obese individuals are at risk for developing cardiovascular disease due to elevated



blood lipids, following a long-term low fat diet would not only help prevent cardiovascular risk, but may also reduce the need for blood lipid lowering medication.

The role of the glycemic index (GI) in improving insulin sensitivity as well as reductions in IMCL improving insulin resistance has been documented. However, it is uncertain whether increased IMCL storage is a cause or consequence of insulin resistance. In the second study of this thesis, the data did not support the hypothesis that a low glycemic index (GI) diet would reduce IMCL stores, thereby improving insulin sensitivity in adults with abdominal obesity. The lack of significant findings may be due to the minimal change of -6.9% in GI for the low GI diet, as well as other issues associated with dietary studies such as accuracy in recording of 3-day food diaries and weekly test food record diaries, and diet compliance. This study demonstrated that the change in diet GI did not reduce fasting glucose but did reduce 60 minute postprandial glucose and only reduced 120 minute postprandial glucose in individuals who already had high glucose. Therefore the lack of effect on IMCL may be that the reduction in glucose was not large enough or that it takes longer than 24 weeks to see an effect. A limitation of this study was that the insulinogenic index and HOMA index was used to measure insulin sensitivity, whereas the euglycemic hyperinsulinemic clamp technique is considered the reference standard to measure insulin mediated glucose disposal and insulin sensitivity (8). Although the HOMA index has been validated with the euglycemic hyperinsulinemic clamp technique (9), employing the euglycemic hyperinsulinemic clamp technique in a long-term study may provide further information on the role of the GI and IMCL in improving insulin sensitivity.

Research has demonstrated that the GI predicts postprandial glucose responses elicited by mixed meals (10, 11, 12, 13) in participants under controlled conditions. The question about whether the GI can predict individual glycemic responses to self-selected meals remains unclear.

To address this issue, a continuous glucose monitoring system (CGMS) was used to assess glycemic responses of a breakfast meal in the third study. It was hypothesized that the GI would be a significant determinant of individual glycemic responses when individuals self-selected their breakfast meals. The hypothesis was supported with the GI being the greatest determinant of individual glycemic responses to a breakfast meal containing carbohydrate, fat and protein. The results of our study in abdominally obese adults were in agreement with Fabricatore et al (14) who also addressed this issue in overweight and obese, type 2 diabetics. These results support the validity of the GI and that following a low GI diet can be beneficial in controlling blood glucose in obese individuals with or without diabetes.

## **7.2 Future Directions**

Both this study and research by Goff et al (15) have not shown changes in IMCL with a low GI diet due to the minimal change in GI. Future long term studies are needed with a greater change in GI to determine whether dietary GI will reduce IMCL thereby improving insulin sensitivity. Future studies could also include different populations, especially individuals with insulin resistance or type 2 diabetes, since they would generally have elevated IMCL at baseline.

## **7.3 Conclusions**

The main hypothesis of this study was a low glycemic index (GI) diet will reduce intramyocellular lipid (IMCL) stores, thereby improving insulin sensitivity in weight-stable adults with abdominal obesity. The data presented in this dissertation do not support this hypothesis. However, IMCL was positively correlated with the HOMA index concluding that there is a positive relationship between IMCL and insulin resistance. It was also concluded that a low GI diet improves 60 minute postprandial glucose and lowers 120 minute postprandial glucose in abdominally obese individuals with high blood glucose.

The hypothesis for the run-in phase of the main study was following dietary advice on lowering overall fat and saturated fat intake will improve metabolic profiles in adults with abdominal obesity. The data presented in this dissertation support this hypothesis with respect to total cholesterol and LDL cholesterol. It was concluded that a low fat, weight maintaining diet significantly reduces total cholesterol and LDL cholesterol.

The third hypothesis was the glycemic index (GI) is a significant determinant of individual glycemic responses elicited by self-selected breakfast meals in free-living, abdominally obese adults. The data presented in this dissertation support this hypothesis. It was also concluded that GI is a more important determinant of glycemic response than available carbohydrate intake.

## 7.4 References

1. James PT, Leach R, Kalamara E, Shayeghi M. The worldwide obesity epidemic. *Obesity Research* 2001;9:228S–233S.
2. Balkau B, Deanfield JE, Despres JP, Bassand JP, Fox, KAA, Smith SC Barter P, Tan C, Van Gaal L, Wittchen HU, Massien C, Haffner SM. International day for the evaluation of abdominal obesity (IDEA): A study of waist circumference, cardiovascular disease, and diabetes mellitus in 168 000 primary care patients in 63 countries. *Circulation* 2007;116:1942-1951.
3. Machann J, Haring H, Schick F, Stumvoll M. Intramyocellular lipids and insulin resistance. *Diabetes, Obesity and Metabolism* 2004;6:239-248.
4. Gastaldelli A, Natali A, Vettor R, Corradini SG. Insulin resistance, adipose depots and gut: Interactions and pathological implications. *Digestive and Liver Disease* 2010;42:310-319.
5. National Cholesterol Education Program National Heart, Lung, and Blood Institute. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), Final Report, National Institutes of Health. NIH Publication No. 02-5215, September 2002.
6. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, Loviscach M, Stumvoll M, Claussen CD, Schick F, Haring HU, Jacob S. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 2001;50:2579-2584.
7. Schrauwen-Hinderling VB, Kooi ME, Hesselink MKC, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie G, Saris WHM, Nicolay K, Schrauwen P. Intramyocellular lipid content

- and molecular adaptations in response to a 1-week high-fat diet. *Obesity Research* 2005;13:2088-2094.
8. American Diabetes Association. Consensus development conference on insulin resistance. 5-6 November 1997. *Diabetes Care* 1998;21:310-314.
  9. Saad MF, Anderson RL, Laws A, Watanabe RM, Kades WW, Chen YDI, Sands RE, Pei D, Savage PJ, Bergman RN. The Insulin Resistance Atherosclerosis Study: Comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. *Diabetes* 1994;43:1114-1121.
  10. Brynes AE, Adamson J, Dornhorst A, Frost GS. The beneficial effect of a diet with low glycaemic index on 24 h glucose profiles in healthy young people as assessed by continuous glucose monitoring. *British Journal of Nutrition* 2005;93:179-182.
  11. Wolever TMS, Yang M, Yi Zeng X, Atkinson F, Brand-Miller JC. Food glycemic index, as given in Glycemic Index tables, is a significant determinant of glycemic responses elicited by composite breakfast meals. *American Journal of Clinical Nutrition* 2006;83:1306-1312.
  12. Brynes AE, Lee JL, Brighton RE, Leeds AR, Dornhorst A, Frost GS. A low glycemic diet significantly improves the 24-h blood glucose profile in people with type 2 diabetes, as assessed using the continuous glucose MiniMed monitor. *Diabetes Care* 2003;26:548-549.
  13. Nansel TR, Gellar L & McGill A. Effect of varying glycemic index meals on blood glucose control assessed with continuous glucose monitoring in youth with type 1 diabetes on basal-bolus insulin regimens. *Diabetes Care* 2008;31:695-697.
  14. Fabricatore AN, Ebbeling CB, Wadden TA, Ludwig DS. Continuous glucose monitoring to assess the ecologic validity of dietary glycemic index and glycemic load. *American Journal of Clinical Nutrition* 2011;94:1519-1524.

15. Goff LM, Frost GS, Hamilton G, Thomas EL, Dhillon WS, Dornhorst A, Bell JD.

Carbohydrate-induced manipulation of insulin sensitivity independently of intramyocellular lipids. *British Journal of Nutrition* 2003;89:365-374.

**CHAPTER 8**  
**APPENDICES**

## **APPENDIX 8.1**

### **PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT**



## PARTICIPANT INFORMATION SHEET

**Title of Study:** A randomized, 2X2 factorial design study to evaluate the effects of telmisartan versus placebo, and of a low-glycemic index diet versus a low-fat diet, in reducing intra-myocellular lipid content in people with the metabolic syndrome (TRIM Study).

**Investigator:** Dr. A. M. Sharma  
 Professor of Medicine  
 Center for Cardiovascular Obesity Research and Management  
 McMaster University, Faculty of Health Sciences.

**Sponsor:** Canadian Institutes of Health Research (CIHR)  
 Boehringer Ingelheim Canada Ltd. /Ltee.  
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## INTRODUCTION

You are being invited to participate in a research study conducted by Dr. Arya M. Sharma, because you may have signs of the “metabolic” syndrome”, which is characterized by the presence of abdominal obesity, high blood pressure, glucose, and/or lipid (fat) abnormalities.

In order to decide whether you want to be a part of this research study, you should understand what is involved and the potential risks and benefits. This form gives detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate. Please take your time to make your decision. Feel free to discuss it with your friends and family, or your family physician.

McMaster University and the investigator Dr. Arya M. Sharma are under contract with the Sponsors of this study and are receiving compensation to cover the costs of conducting the study. Dr. Sharma has previously conducted research funded by Boehringer Ingelheim, and has received honoraria from this company both as a consultant and as a speaker on topics related to the use of telmisartan (Micardis®) at scientific meetings sponsored by Boehringer Ingelheim.

## WHY IS THIS RESEARCH BEING DONE?

One out of two adults in Canada are currently overweight and therefore at increased risk for a number of medical conditions including diabetes, heart attacks and stroke. This increased risk is often due to the presence of the so-called “metabolic syndrome”, which is characterized by the presence of high blood pressure, blood glucose and/or lipid (fat) abnormalities. This research is being done to better understand the factors underlying the metabolic syndrome and to determine whether this syndrome can be improved by treatment with a drug (telmisartan- Micardis®) and/or a “low-glycemic” diet.

## WHAT IS THE PURPOSE OF THIS STUDY?

People with the metabolic syndrome are at increased risk for diabetes and heart disease because they do not respond well to insulin. This condition is called insulin resistance. Insulin is a hormone that allows sugar to enter muscle cells. People with the metabolic syndrome may be insulin resistant because they have increased muscle fat, i.e. they store large amounts of fat in their muscle cells. Many factors like diet, exercise, fat tissue, liver function, and genetics can affect muscle fat.

Some drugs can also influence muscle fat. Telmisartan belongs to a class of drugs called “angiotensin receptor blockers” commonly used for the treatment of high blood pressure. Laboratory experiments have shown that these drugs may also affect the growth of fat cells and may therefore influence the storage of fat in other organs like muscle or liver. These drugs may also reduce insulin resistance. This could be of benefit to people with the metabolic syndrome. One aim of this study is therefore to examine the effect of telmisartan on muscle fat, insulin sensitivity and fat tissue in people with this syndrome.

Dietary factors can also influence muscle fat and how the human body responds to insulin. Reducing the amount of easily digested carbohydrates in the diet (a “low-glycemic” diet) can improve how the body responds to insulin and may have positive effects on blood sugar and fat levels. The mechanism of this effect is not known. Therefore, a second purpose of this study is to examine whether a low-glycemic diet will also affect muscle fat.

The effect of telmisartan and a low-glycemic diet on muscle fat will be tested in about 100 people with the metabolic syndrome. A research ethics board that ensures that research studies do not violate an individual’s rights has reviewed and approved this study protocol.

## WHO CAN PARTICIPATE IN THIS STUDY?

You may be eligible to participate in this study if you have at least three features of the metabolic syndrome. These include increased waist circumference, elevated blood glucose, abnormal blood fats, and/or elevated blood pressure. You must also be willing to follow the dietary and treatment protocol required by the study and to complete the various visits and investigations required by the protocol. As a number of medications can interfere with the study, you **cannot** take part in this study if you are currently on certain medications that can affect your blood pressure or blood sugar levels.

Because one of the main tests involved in the study will be performed by magnetic resonance spectroscopy (MRS), you **cannot** participate in this study if you have any contraindications to this technique, e.g. claustrophobia (fear of closed spaces), metallic body parts, pacemakers, clipped blood vessels, or metallic fragments in your eye.

## HOW LONG WILL THE STUDY LAST

The study will last about 32 weeks and requires at least 16 visits. In addition there will be 6-8 visits for special examinations during the course of the study. The total time for your participation in this study is estimated to be around 40 – 50 hours.

## HOW WILL I BE ASSIGNED TO A TREATMENT GROUP IN THIS STUDY?

The participants in the study will be assigned at random, that is, by a method of chance (like a flip of a coin), to one of four groups. You will have a 1 in 4 chance of being in one of the following groups:

- 1) Telmisartan and low-glycemic diet.
- 2) Placebo and low-glycemic diet.
- 3) Telmisartan and control diet.
- 4) Placebo and control diet.

In this study you have a 50% chance of receiving telmisartan and a 50% chance of receiving the placebo. A placebo is an inactive substance, like a sugar pill. The study medication will be taken once per day in the morning, with or without food, for the duration of the study. Neither you nor your study doctor will know which group you will be in. In an emergency, the randomization code can be broken. You will also have a 50% chance of being in the group that receives the low-glycemic diet or the control diet. The control diet consists of a low-fat diet, as recommended by the American Heart Association for the treatment of individuals with high blood fat levels. Assignment to either diet is open, so both you and your doctor will know whether you are on the low-glycemic or control diet. Despite being on telmisartan, placebo, or either diet, it is possible that your metabolic syndrome may not improve or may worsen. Your condition will be carefully monitored. If it does worsen, the study doctor will determine whether additional treatment is necessary and whether it is safe or unsafe for you to continue in the trial.

## WHAT TYPE OF A DIET WILL I NEED TO FOLLOW IF I TAKE PART IN THE STUDY?

If you participate in this study, at visit 2 you will be asked to follow a low fat diet. This diet is recommended by the American Heart Association for people with high blood fat levels. In addition to the low fat diet, at Visit 6 you will be assigned to either the low-glycemic diet or control diet, by chance (like flipping a coin). If you are assigned to the low-glycemic diet you will be asked to eat a certain amount of carbohydrates that are less easily digested (e.g. whole wheat bread, whole wheat pasta, and legumes). If you are assigned to the control diet, you will be asked to eat a certain amount of carbohydrates that are easily digested (e.g. white bread, rice, baked potato). Some key foods, which will make it easier for you to follow these diets, will be provided to you in the study. You will also be given instructions on how to choose other specific foods that you need to eat.

At most visits the nutrition counsellor will meet with you to review your dietary habits, give advice, and have you complete a MedFICTS questionnaire. The MedFICTS questionnaire is a diet assessment tool that is used to assess your Calorie and fat intake. Along with the key foods you will be given a daily food record to keep track of their use and you will be asked to complete and return them prior to visits 7, 9, 10, 11, 12 and 14. These key food records will be reviewed at these visits. You will also periodically be given a 3-day food diary to complete and return before future visits. In these diaries, you will be asked to record your dietary intake and physical activity habits. These diaries will be explained to you and you will be asked to return them before visits 2, 6, 7, 10 and 14. The diaries will be reviewed at these visits.

Potential Risks: There are no harmful effects with either diet.

Potential Benefits: You will receive dietary counselling and learn how to keep accurate records of your food intake and activity habits. You will not be expected to lose weight on either diet. However, reducing your fat intake may result in some weight loss (3 to 5 pounds) in some individuals.

## WHAT WILL MY RESPONSIBILITIES BE IF I TAKE PART IN THE STUDY?

If you participate in this study, you will be asked to come to a **screening visit (first visit)** after fasting for 12 hours (no alcohol for 24 hours). Fasting is when you do not have anything to eat or drink except water. If you do not fast before visits that require a fast, you will have to return at another time when you have fasted. At the screening visit you will: a) be asked about previous medical problems, your current health and your medications; b) have a brief examination including your weight, height, blood pressure, heart rate, waist/hip circumference; c) have an electrocardiogram (a simple test that measures the electrical activity of the heart and that can identify damage to the heart muscle); d) have a bioelectrical impedance analysis (a simple test that estimates body water content by passing a low-voltage current through the body); e) You will also have blood drawn (3 tablespoons) to check your blood sugar levels, kidney and liver function, and blood count and fat levels; f) supply a random urine sample to check kidney function; and g) You will also be given a 3-day food diary to complete and return before

the next visit. If you are a woman who is able to become pregnant the urine sample will also be used to do a pregnancy test and you will be asked about your contraceptive methods or childbearing potential. *This visit should take between 2 and 3 hours.*

At the **second visit** you will be seen by a physician or a nurse who will review the screening blood test results, ECG, and your medication. Your weight, blood pressure, and heart rate will be measured. The physician or nurse will determine whether you can be enrolled in the study. If you meet the initial eligibility criteria, you will be given the “run-in” study medication and be asked to take the first dose in the office. You will be instructed on how to take your medication. You will also meet with the nutrition counsellor to review your three-day food diary, and you will also complete a MedFICTS questionnaire and be instructed on starting and maintaining the run-in diet. *This visit should take between 1 and 2 hours.*

**One week after the second visit**, the nutrition counsellor will telephone you to assess your compliance with the study diet and provide dietary advice.

The **third visit** you will have your weight, blood pressure and heart rate monitored. The study nurse will review your compliance with the run-in medication, your medication, monitor adverse events, and you will be given run-in medication. You will then meet with the nutrition counsellor to complete a MedFICTS questionnaire, assess your compliance with the diet, and be provided with dietary advice including any dietary adjustments or changes that may be required. *This visit should take 1-2 hours.*

At the **fourth visit** the study nurse will review your compliance with the run-in medication, your medication, monitor adverse events, measure weight, blood pressure, and heart rate. You will then meet with the nutrition counsellor to complete a MedFICTS questionnaire, assess your compliance with the diet, and be provided with dietary advice including any dietary adjustments or changes that may be required (similar to third visit). You will be given a 3-day food diary to complete and bring back with you at the sixth visit. *This visit should take around 1-2 hours.*

The **fifth visit** includes a series of baseline tests that will be completed before you are assigned to a treatment group. These tests will be scheduled over a 2 week period and will not be completed on the same day. In order to minimize your number of visits, some of these tests may be scheduled with Visit 4 and/or visit 6. Due to scheduling availability, you will not have these tests in any particular order. The baseline tests include: magnetic resonance spectroscopy (MRS), HOMA/OGTT/biomarkers/lipid profile (fasting), bioelectrical impedance analysis (BIA), fat biopsy (fasting), muscle biopsy (4 hour fast), Doppler ultrasound of the brachial artery in forearm (fasting), echocardiography, continuous subcutaneous glucose monitoring, and Actical physical activity monitor. With the OGTT you will have blood drawn (9 tablespoons) and your weight, blood pressure and heart rate will be measured. *This visit will take a total of about 9-10 hours (not consecutive hours).*

At the **randomization (sixth) visit** you will bring a completed 3-day food diary (given to you at visit 4). The study nurse will review your compliance with the run-in medication, monitor adverse events, review your medication, check biopsy and puncture sites and review your eligibility for the study. You will then meet with the nutrition counsellor who will assess your dietary compliance. If you are determined to be compliant with the run-in medication and the run-in diet you will be randomized to one of four study groups. Your weight, blood pressure, heart rate, and waist/hip circumference will be measured. You will be asked to provide a random urine sample that will be stored until analysis (kidney function test). If you are a pre-menopausal woman, a pregnancy test will be performed on the urine sample. If you are on low-dose aspirin, an additional sample of your urine will be stored for until analysis. You will be given the study medication and will be asked to take the first dose at this visit. You will then meet with the nutrition counsellor to complete the MedFICTS questionnaire, get dietary instructions, and be given test foods. The nutrition counsellor will instruct you on how to maintain the weekly test-food records. You will also be given a 3-day food diary to complete and return at the seventh visit. *This visit should take around 1-2 hours.*

At the **seventh visit**, you will come in with a completed weekly test-food record and 3-day food diary. The study nurse will review compliance with the study medication, monitor adverse events, review your medications, measure your weight, blood pressure and heart rate. At this visit, your dose of study medication (telmisartan or placebo as per assigned study arm) will be increased from 80mg to 160mg (up-titrated). You will be given the study medication and then asked to take the first uptitrated dose at this visit. You will also meet with the nutrition counsellor to complete the MedFICTS questionnaire, assess your compliance with the diet, be provided with dietary advice including any dietary adjustments or changes that may be required, and be given test foods. You will be given weekly test food records for you to complete and return at the ninth visit. *This visit should take around 1-2 hours.*

At the **eighth visit** the study nurse will review compliance with the study medication, review your medications, monitor adverse events, measure weight, blood pressure and heart rate, and draw a blood sample (1 teaspoon) to assess your kidney function. If at this visit your kidney function blood tests have increased by more than 30% since the beginning of the study, you will be asked to lower the dose of study medication to 80mg (telmisartan or placebo) and an unscheduled visit will be scheduled to repeat the kidney function blood tests (1 teaspoon). If the repeated blood test results remain abnormal, the study doctor will decide if it is unsafe for you to continue the study medication, even at the lower dose. If so, the study medication will be discontinued but you can remain enrolled in the dietary arm of the study. If the repeated blood tests are normal you will continue the study medication, but at the lower dose. *This visit should take between 1-2 hours.*

At the **ninth visit** you will come in with a completed weekly test food record. The study nurse will review compliance with study medication, review your medications, monitor adverse events, measure your weight, blood pressure and heart rate, and give you study medication. You will also meet with the nutrition counsellor to complete the

MedFICTS questionnaire, assess your compliance with the diet, be provided with dietary advice including any dietary adjustments or changes that may be required, and be given test foods. You will be given weekly test food records and a 3-day food diary for you to complete and return at the tenth visit. *This visit should take around 1-2 hours.*

At the **tenth visit** you will come in after an overnight (12hour) fast (no alcohol for 24 hours) and bring in a completed 3-day food diary and the weekly test-food records. The study nurse will review compliance with study medication, review your medications, monitor adverse events, measure your weight, blood pressure, heart rate, and waist/hip circumference, and perform a bioelectrical impedance analysis. You will be asked to provide a random urine sample that will be stored until analysis (kidney function test). If you are a pre-menopausal woman, a pregnancy test will be performed on that urine sample. You will have the OGTT at this visit with blood samples drawn (approximately 5 tablespoons) for analysis of blood sugar levels, insulin and biomarkers. The study nurse will give you study medication. You will also meet with the nutrition counsellor to complete the MedFICTS questionnaire, assess your compliance with the diet, be provided with dietary advice including any dietary adjustments or changes that may be required, and be given test foods. You will be given weekly test food records for you to complete and return at the eleventh visit. *This visit should take around 3 hours.*

At the **eleventh visit** you will come in with a completed weekly test-food record. The study nurse will review compliance with the study medication, review your medications, monitor adverse events, measure your weight, blood pressure and heart rate, and give you study medication. You will also meet with the nutrition counsellor to complete the MedFICTS questionnaire, assess your compliance with the diet, be provided with dietary advice including any dietary adjustments or changes that may be required, and be given test foods. You will be given weekly test food records for you to complete and return at the twelfth visit. *This visit should take around 1-2 hours.*

At the **twelfth visit** you will come in with a completed weekly test-food record. The study nurse will review compliance with the study medication, review your medications, monitor adverse events, measure your weight, blood pressure and heart rate, and give you study medication. You will also meet with the nutrition counsellor to complete the MedFICTS questionnaire, assess your compliance with the diet, be provided with dietary advice including any dietary adjustments or changes that may be required, and be given test foods. You will be given weekly test food records and a 3-day food diary for you to complete and return at the fourteenth visit. You will then be scheduled for the end of study tests (visit 13). *This visit should take 1-2 hours.*

The **thirteenth visit** includes a series of end of study tests that will be completed before you discontinue study medication and study diet. These tests will be scheduled over a 2 week period and will not be completed on the same day. In order to minimize your number of visits some of these tests may be scheduled with Visit 12 and/or visit 14. Due to scheduling availability, you will not have these tests in any particular order. The end of study tests include: magnetic resonance spectroscopy (MRS), HOMA/OGTT/biomarkers/lipid profile (fasting), bioelectrical impedance analysis (BIA),

fat biopsy (fasting), muscle biopsy (4 hour fast), echocardiography, continuous subcutaneous glucose monitoring, and Actical physical activity monitor. With the OGTT you will have blood drawn (9 tablespoons) and your weight, blood pressure and heart rate will be measured. *This visit will take a total of about 9-10 hours (not consecutive hours).*

The **fourteenth visit** you will bring in a completed 3-day food diary and weekly test-food records (given to you at visit 12). The study nurse will review compliance with the study medication, review your medications, monitor adverse events, measure your weight, blood pressure and heart rate, and you will have a physical examination. You will be asked to provide a random urine sample that will be stored until analysis (kidney function test). If you are a pre-menopausal woman, a pregnancy test will be performed on the urine sample. If you are on low-dose aspirin, an additional sample of your urine will be stored until analysis. You will be instructed to stop taking the study medication (all remaining medication will be collected). You will also meet with the nutrition counsellor to complete the MedFICTS questionnaire, review dietary compliance and weekly test-food records. *This visit should take around 1-2 hours.*

The **fifteenth visit** you will be scheduled three days to one week following discontinuation of study medication. You will have a Doppler ultra sound of the brachial artery in the forearm (to assess your blood vessel functioning). *This visit should take around 1 hour.*

The **sixteenth visit** will be a follow-up telephone call 2 weeks after stopping study medication, to discuss with you any remaining issues regarding your participation in the study. The study nurse will assess adverse events and schedule a clinic visit if necessary.

## WHAT TESTS WILL I HAVE AND WHAT ARE THE BENEFITS OR RISKS OF THESE TESTS?

### Blood Tests

Blood will be drawn at Visits 1, 5, 8, 10 and 13 to test your blood count, blood sugar, blood fats, insulin and other hormones, markers of kidney and liver function, markers of inflammation, obesity and heart problems. The amount of blood drawn at each visit will range between 5 cc (1 teaspoon) to about 135 cc (9 tablespoons). The total amount of blood taken over the course of the study will be around 400 cc, which is less than that taken at a normal blood donation and should have no negative effects. Your blood samples will be sent to and stored in the Research Laboratory at the Hamilton General Hospital (HRLMP Clinical Trials and Clinical Research) and in Dr. Sharma's lab at McMaster University in line with approved procedures. Your blood samples will be used for the purpose of this study and will allow us to explore new risk factors for obesity as they emerge in the future.



Potential risks: There is a possibility of a small bruise at the site where the needle stick is administered. Some individuals may also experience a feeling of fainting on having their blood drawn. Please inform the study nurse if you have previously experienced such a reaction.

Potential benefits: These tests will provide information on your blood glucose and fat levels, which, if elevated, are important risk factors for heart disease and stroke. These tests will also provide information on kidney and liver function. Should any abnormalities be detected in these tests, they will be discussed with you by the study doctor who will discuss the importance and treatment options for these findings.

### Glucose Tolerance Test

An oral glucose tolerance test will be performed at Visits 5, 10 and 13. For this test we will insert an intravenous needle (a small, 2 inches long, plastic needle as used for infusions) into your vein for drawing blood samples. After taking 3 timed blood samples (a total of 105 cc or 7 tablespoons), you will drink a bottle of sweet liquid containing 75 g of glucose (sugar) and have 10 cc (2 teaspoons) of blood drawn after 30, 60, and 120 min. This test, commonly used for the diagnosis of impaired glucose tolerance and diabetes, will be used to determine your glucose and insulin responses to a glucose load. These variables will be used to calculate your sensitivity to insulin and the rate of insulin release from your pancreas.

Potential Risks: Some individuals may feel nausea on drinking the sweet liquid. There are no harmful effects.

Potential benefits: This will provide information on your risk for diabetes and heart disease. Should any abnormalities be detected, they will be discussed with you by the study doctor who will discuss the importance and treatment options for these findings.

### Magnetic Resonance Spectroscopy

This test will be performed to examine your muscle, liver and visceral (internal organs) fat at Visit 5 and Visit 13. The Magnetic Resonance Spectroscopy (MRS) is a new method of scanning that examines structures in the body using magnetic fields and harmless low energy radio waves. No x-rays are used. The scanning process is totally painless and has no known harmful effects. However some people may not be suitable for MRS scanning so you will be required to fill in a patient questionnaire before each procedure.

The scan can take from 30 to 60 minutes. During the scan you will be asked to lie on a special table, which moves into the tunnel of the machine. You need to keep still during the examination and at times you will be asked to hold your breath. The technician will talk to you through an intercom telling you what is happening, and when they are starting each set of new scans. You will have verbal contact with the operator and the

use of a button that will allow you to alert the operator if you need to be taken out of the machine.

At times the machine makes loud knocking noises, which is part of the normal working of this machine. You can wear earplugs or listen to music played in the background to make this noise less obvious.

This test will be performed at McMaster University Medical Center. This test will be done in the evening hours or during a weekend.

Potential Risks: The scanning process is totally painless and has no known harmful effects. However, some people may find lying in the scanner difficult to tolerate, as the tunnel is quite narrow. If this should occur, imaging will be stopped immediately, and you will be removed from the scanner.

Potential Benefits: Currently, this test is mainly used for scientific investigations and the results of this test are unlikely to be of direct benefit to you. However, if the scanning does show an undiagnosed condition you will be informed and adequate therapy recommended.

#### Fat Biopsy (mini liposuction)

From all participants of this study, a total two fat biopsies will be taken from the area around the navel (one at Visit 5 and one at Visit 13). This procedure involves the removal of small pieces of fat tissue using a sterile hollow needle, a technique also referred to as mini liposuction. A medical doctor will clean an area located a few inches around your navel and inject a small amount of local anaesthetic ("freezing") into and under the skin. He/she will then make a small incision (~4-5 mm) in the skin in order to create an opening through which to put the biopsy needle into the fat tissue located under your skin. There is a small amount of bleeding from the incision, but this is minimal. The doctor will then loosen and collect small pieces of fat through the biopsy needle. This procedure will be repeated several times to ensure that enough fat is collected (~2-5 grams; about 1-2 teaspoons). During the time that the samples are being taken (~2-3 minutes), you may feel the pressure of the needle and on some occasions this may be moderately painful. However, the discomfort passes very quickly. This test will be performed at the Hamilton General Hospital. The total time for each biopsy is around 20-30 minutes.

Following the biopsies, the incisions will be closed with a sterile strip (paper tape) and covered with a sterile dressing. In order to allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided for at least 4 days following the biopsy procedure.

Potential Risks. The biopsy technique is routinely used in physiological research, and complications are rare provided that proper precautions are taken. However, there may be some internal bleeding at the site of the biopsy, which can result in bruising and temporary discoloration of the skin. On occasion, a small lump may form under the site of the incision, but this normally disappears within 2-3 months or within a few days to a week if massaged. As with any incision, there is also a slight risk of infection, however this risk is virtually eliminated through proper cleansing of the area and daily changing of wound coverings. If the incision does not heal within a few days or you are in any way concerned about inflammation or infection, please contact us immediately.

Dr. Sharma, a medical doctor who is trained in this procedure, will perform this biopsy. In past experience with similar subjects, almost all subjects experienced bruising and discoloration of the skin at the site of the biopsy (which lasted about 1-2 weeks); approximately 1 in 400 have experienced a local skin infection; 1 in 20 have experienced a small lump at the site of the biopsy (in all cases this disappeared within about 1-2 weeks using local massage); and 1 in 400 have experienced a temporary loss of sensation in the skin at the site of incision (an area of numbness about the size of a Loonie which lasted up to 4 months). There is also an extremely remote chance (1 in 1,000,000) that you will be allergic to the local anaesthetic.

Potential Benefits: This test is currently only used for scientific investigations and the results of this test are unlikely to be of direct benefit to you. However, this test can help us better understand the effects of telmisartan and the low-glycemic diet on fat tissue biology.

## Muscle Biopsy

From some participants of this study, a total of two muscle biopsies will be taken from the outer thigh (one at Visit 5 and one at Visit 13). This procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle. A medical doctor will clean an area over your quadriceps muscle (Vastus Lateralis) and inject a small amount of local anaesthetic ("freezing") into and under the skin. He/she will then make a small incision (~4-5 mm) in the skin in order to create an opening through which to put the biopsy needle into your thigh. There is a small amount of bleeding from the incision, but this is minimal. The doctor will then quickly cut off a very small piece of muscle (~250 mg; about the size of the eraser on the end of a pencil) and remove the needle from your leg. A small sample of fat tissue will also be collected from this site at the same time. During the time that the sample is being taken (~5 sec), you may feel the sensation of deep pressure in your thigh and on some occasions this is moderately painful. However, the discomfort very quickly passes and you will be quite capable of performing exercise and daily activities.

Following the biopsies, the incisions will be closed with sterile suture (stitch), and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the anaesthetic wears off, your leg may feel tight and often

there is the sensation of a deep bruise or "Charlie Horse". Analgesics (pain killers) such as Tylenol ® or Ibuprofen (such as Advil ® or

Motrin ®) are acceptable if you experience significant pain associated with the biopsy. It is also beneficial to periodically apply an ice pack to the biopsy site the following day, as this will help to reduce any swelling and any residual soreness. The following day your leg may feel uncomfortable when going downstairs. The tightness in the muscle usually disappears within 2 days and subjects routinely begin exercising at normal capacity within a day. In order to allow the incision to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided for at least 4 days following the biopsy procedure.

This test will be performed at McMaster University Medical Center. The total time for each biopsy is around 30-60 minutes.

Potential Risks. The biopsy technique is routinely used in research, and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in bruising and temporary discoloration of the skin. On occasion, a small lump may form under the site of the incision, but this normally disappears within 2-3 months or within a few days to a week if massaged. As with any incision, there is also a slight risk of infection, however this risk is virtually eliminated through proper cleansing of the area and daily changing of wound coverings. If the incision does not heal within a few days or you are in any way concerned about inflammation or infection, please contact us immediately.

This biopsy will be performed by Dr. Tarnopolsky, who has performed over 10,000 of these in patients and healthy people ranging in age from 1 week to 90 years. In past experience with healthy young subjects, approximately 1 in 2,000 have experienced a local skin infection; 1 in 500 have experienced a small lump at the site of the biopsy (in all cases this disappeared within ~1-2 weeks using local massage); 1 in 1,500 have experienced a temporary loss of sensation in the skin at the site of incision (an area of numbness about the size of a Loonie which lasted up to 4 months), and 1 in 100 have experienced bruising around the site of incision which lasted for ~4-5 days. While there is also a theoretical risk of damage to a small motor nerve (that is used to allow your muscle to move) branch of the outer leg muscle, this has never been seen in over 10,000 biopsies performed by Dr. Tarnopolsky. The risk of damaging a small motor nerve branch is impossible to truly estimate, but in the extremely unlikely chance that it did occur, only about 20% of the lower part of one of four large muscles that moves the knee would be affected (hence, it would not impact on function in daily activities) and even this small area of muscle would likely recover in 6 – 9 months. There is also an extremely remote chance (1 in 1,000,000) that you will be allergic to the local anaesthetic.

Potential Benefits: This test is currently only used for scientific investigations and the results of this test are unlikely to be of direct benefit to you. However, this test can help

us better understand the effects of telmisartan and the low-glycemic diet on muscle tissue biology.

You will not be eligible for muscle or fat biopsies if you are taking certain medications such as anti-coagulants (blood thinners), e.g. warfarin, or antiplatelet medication (medication to prevent clumping of certain cells in you blood), e.g. Plavix®. You will be asked about these types of medications at the beginning of the study and before the biopsies are scheduled.

### Continuous Glucose Monitoring

A 3-day blood sugar (glucose) record will be obtained at Visits 5 and 13. For this test a small plastic needle will be inserted under the skin and taped securely. You will feel a small prick when the needle is inserted (most patients find it virtually painless). The needle will then be hooked to a monitor, the size of a mobile phone, which could easily be worn on your belt or placed in your pocket. The monitor will record blood sugars every 5 minutes. You will not feel this.

In order to set the monitor you will need to do finger prick glucose testing 4 times a day using a glucose tester that we will provide. These measurements will then be entered into the monitor.

You can continue all normal activities **except** water immersion sports like swimming, sitting in a hot tub or bathing in a bathtub.

Potential Risks: Besides the mild discomfort that you may experience when the needle is placed under the skin or when you do the finger-prick blood sugar measurements, there is really no other risk to you. Although infection at the insertion site may occur, this is usually not a concern.

Potential Benefits: The 3 day continuous glucose measurements will help us gauge how well you are responding to the diet or medication. This test will also provide more accurate measurements of how your blood sugars vary with activity and meals. If any abnormalities are discovered, the study doctor will discuss these with you.

### Activity Monitoring

In order to monitor your physical activity, you will be asked to wear an electronic activity monitor (Actical™). This monitor is about the size of a matchbox and is worn around your waist on a belt for 72 hours at the same time as the continuous glucose-monitoring device at visits 5 and 13.

Potential Risks: none.

Potential Benefits: This device will let us calculate the amount of calories consumed by physical activity during the measurement period.

## Heart Ultrasound

An ultrasound study of your heart will be performed between visits 4 and 6. Measurements will be taken at rest by a trained ultrasound technician. This test uses high frequency waves to create a picture of the heart. The test is painless and will take around 60 minutes.

Potential Risks: none.

Potential Benefits: This test will provide information on the structure and function of your heart. Should we discover any abnormality that may be important to your health; you will be informed and advised to take the appropriate steps for further diagnostics or treatment.

## Ultrasound of the Blood Vessels of the Arm

In this test, we will measure the diameter of your artery that supplies blood to your arm. This will be done with ultrasound. Measurements are taken at rest, after blocking the circulation in your upper arm with a blood pressure cuff for 5 minutes and after taking one tablet of nitro-glycerine (0.15mg or 0.3mg) under your tongue.

Potential Risks: During this test, a blood pressure cuff will be inflated to high pressures for a continuous 5-minute period. At this time, the blood pressure cuff may cause mild discomfort or aching around your arm. You may feel increasing numbness or tingling (pins and needles) in your hand, which will rapidly stop when the cuff pressure is released. No harmful consequence is expected from this test. The ultrasound should not cause any discomfort. Nitro-glycerine may cause a brief headache or dizziness.

Potential Benefits: This test is currently only used for scientific investigations and the results of this test are unlikely to be of direct benefit to you. However, this test can help us better understand the effects of telmisartan and the low-glycemic diet on blood vessel function.

## Body Composition Analysis (Bioelectrical impedance)

This test will be performed at Visits 1, 5, 10 and 13. This test examines the water and fat content of your body by passing weak electrical current through your body via electrodes attached to your hands and feet. This test is totally painless, has no known harmful effects and takes less than 10 minutes.

## Electrocardiogram (ECG)

An ECG will be performed at Visit 1 or 2. This test measures the electrical activity in the heart through 12 leads connected to your chest and limbs to an electrocardiograph. It is a non-invasive and safe procedure taking less than 15 minutes.

## WHAT WILL HAPPEN WITH MY BLOOD, URINE, AND TISSUE SAMPLES?

All samples will be used for the purpose of this study. All samples will be stored under appropriate conditions either at the Research Laboratory at the Hamilton General Hospital (HRLMP Clinical Trials and Clinical Research) or in the research laboratory of Dr. Sharma. They may also be shared with colleagues at other laboratories for measurements relevant to the study. All samples will be stored and analyzed in a manner that will not allow direct identification of you (the participant). Only Dr. Sharma will maintain a list linking the samples to an individual participant.

Because these tests will be performed for research purposes only and will allow us to explore new risk factors for obesity as they emerge in the future, these tests will not be useful for the diagnosis or management of any medical condition. Therefore you will not be provided with the results of these tests.

Any samples remaining after the completion of the above measurements will be stored for a maximum duration of 10 years, after which they will be adequately discarded or destroyed. During this time the samples can also be used for the measurements of new tests that may arise and be deemed relevant for this project.

The samples will be used for research and such use may result in inventions or discoveries that create new products or diagnostic or therapeutic agents. In some instances, these inventions and discoveries may be of potential commercial value and may be patented and licensed by the researchers/sponsor. You will not receive any money or other benefits derived from any commercial or other products that may be developed from use of the samples.

## WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

This study will study the effects of telmisartan in people with metabolic syndrome. Telmisartan (MiCARDIS ®) is a commonly used drug for the control of high blood pressure. The major side effects of telmisartan are headache, dizziness, fatigue (tiredness), back pain, diarrhea, and upper respiratory tract infections and sinusitis. In rare cases, a potentially life threatening condition called angioedema may occur. The symptoms of angioedema may include swelling of the face, lips, tongue and throat. If you have any of these symptoms at any time during the study, immediately discontinue study medications and contact your physician or study doctor. As the dose of telmisartan used in this study is higher than the dose approved for the treatment of high blood pressure, the side effects may be more common. This study will also test the effect of a “low-glycemic” or a “low-fat” diet. This requires your cooperation and may be considered inconvenient. There is a reasonable likelihood (1 in 50) that changes to your diet may result in indigestion, constipation, bloating, or other symptoms. In such cases, you should report these problems to the nutrition counsellor, who will recommend appropriate adjustments to your diet.

The potential risks and discomforts of the various tests have been described above. When blood samples and biopsies are taken, you may have some discomfort and/or develop some bruising or very rarely, a minor infection. You may also have some nausea during the oral glucose tolerance tests. You will be asked about side effects of the diet and treatment at each visit.

Because the safety of telmisartan for an unborn fetus or newborn is unknown, you cannot participate in this study if you intend to become pregnant, are pregnant or are breastfeeding. If you are a woman who is able to have children, you must agree not to become pregnant while you are in this study. You will need to use an acceptable method of birth control to avoid pregnancy. Acceptable methods of birth control for this study include: intra-uterine device (IUD), oral, implantable/injectable contraceptives, or epidermal patch. Using barrier methods such as condoms, vaginal diaphragm, spermicidal jelly or sponge is not acceptable. If you become pregnant despite these precautions OR if your method of birth control is discontinued or changes, you agree to immediately notify the study team.

If you choose to take part in this study, you will be told about any new information, which might affect your willingness to continue to participate in this research.

#### HOW MANY PEOPLE WILL BE IN THIS STUDY?

There will be at least 100 people with the metabolic syndrome taking part in this study.

#### WHAT ARE THE POSSIBLE BENEFITS FOR ME AND/OR FOR SOCIETY?

The information obtained from participating in this study may show that telmisartan and/or a low-glycemic diet are effective in the reduction of muscle fat and improve insulin sensitivity and other health problems related to the metabolic syndrome. Observations made in this study may also help us better understand some of the biological abnormalities related to this syndrome. No other benefit of participation can be guaranteed.

You will receive some counselling regarding healthy lifestyles and will receive all of your study medication and some foods free of charge. You will also receive information regarding your body composition, blood pressure, blood glucose and lipid values and heart function during the course of the study.

#### IF I DO NOT WANT TO TAKE PART IN THE STUDY, ARE THERE OTHER CHOICES?

It is important for you to know that you can choose not to take part in the study. There are other choices such as improving your diet and increasing your level of exercise or taking other medications, for the treatment of the metabolic syndrome. Your study doctor will discuss these with you.



Choosing not to participate in this study will in no way affect your care or treatment.

#### WHAT INFORMATION WILL BE KEPT CONFIDENTIAL?

The study staff will contact your family doctor regarding your selection and participation in this study. This is important for your safety and well being.

Personal records relating to this study will be kept confidential at all times except where required by law. Only the study doctor will keep a record of your name, address, phone number, health card number and family doctor's name. On documents, your initials and assigned participant number will identify you.

Information obtained during this study, including your medical records, will be available to the sponsor of this study, the Hamilton Health Sciences/McMaster University research ethics board that reviewed the ethical aspects of this study, and the government regulatory agencies (such as Health Canada) to check the accuracy and completeness of the information. This is to ensure that the study has been conducted according to GCP (Good Clinical Practice) quality standards. However, no records, which identify you by name or initials, will be allowed to leave the hospital. By signing this consent form, you or your legally acceptable representative authorizes such access. It is important to note that this original signed consent form and the data, which follows, may be included in your health record.

The results of this study may be published in a professional journal or presented at scientific meetings or to government regulatory authorities; however, your identity will NOT be disclosed in those journals or presentations.

Blood, urine and tissue samples will be identified only by your participant number. The individual results of any analyses, except for the screening and safety tests (blood sugar, blood lipids, blood count, kidney and liver function), body composition and blood pressure will not be made known to you or to any other party and will only be used to identify differences between treatment groups in the study.

Your study records will be stored for the period of time required by applicable law. Until those records and samples are destroyed, you have the right to request that the samples and data collected be destroyed. Confirmation that the samples have been discarded will be made to you in writing.

#### CAN PARTICIPATION IN THE STUDY END EARLY?

If you volunteer to be in this study, you may withdraw at any time and this will in no way affect the quality of care you receive. You may also refuse to answer any questions you do not want to answer and still remain in the study. The investigator may withdraw you from this study if circumstances arise which warrant doing so.

#### WILL I BE PAID TO PARTICIPATE IN THIS STUDY?

A small amount of compensation, will be offered to those patients who qualify for and undergo the muscle and fat biopsies (\$50.00/ biopsy), otherwise, no compensation will be paid for your participation. However, we will reimburse you for parking and meals during your visits.

#### WILL THERE BE ANY COSTS?

Participation in this study will be at no cost to you. The medication, study foods and clinic visits will be provided free of charge. Your participation in this research project will not involve any additional costs to you or your health care insurer.

#### WHAT HAPPENS IF I HAVE A RESEARCH-RELATED INJURY?

Side effects or harm are possible in any research despite high standards of care, and could occur through no fault of your own or the investigators. Known side effects have been described in this consent form, however unforeseeable harm may also occur. If you are injured as a direct result of taking part in this study, you should immediately contact the study doctor. Reasonable and necessary medical expenses will be made available to you by the sponsor (Boehringer Ingelheim), provided such expenses are not covered by your medical or hospital insurance and are in no way attributable to the negligence or misconduct of any agent or employee of McMaster University or Hamilton Health Sciences. Financial compensation for such things as lost wages, disability or discomfort due to this type of injury is not routinely available. However, if you sign this consent form it does not mean that you waive any legal rights you may have under the law, nor does it mean that you are releasing the investigator(s), institution(s) and/or sponsor(s) from their legal and professional responsibilities.

#### IF I HAVE ANY QUESTIONS OR PROBLEMS, WHOM CAN I CALL?

If you have any questions about the study now or later, please phone Sue Damjanovic (TRIM Coordinator) at 905-527-4322 ext. 44710, or if you think you have a research-related injury, please phone Dr. A. Sharma at 905-527-4322 ext 46806 or the Research Nurse at 905-521-5030 (pager).

If you have any questions regarding your rights as a research participant, you may contact: Hamilton Health Sciences Patient Relations Specialist at 905-521-2100, ext. 75240.

## CONSENT STATEMENT

### SIGNATURE OF RESEARCH PARTICIPANT/LEGALLY-AUTHORIZED REPRESENTATIVE

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction. I agree to participate in this study. I understand that I will receive a signed copy of this form.

---

Printed Name of Participant

---

Signature of Participant

---

Date / Time

Consent form administered and explained in person by:

---

Name and title

---

Signature

---

Date / Time

### SIGNATURE OF INVESTIGATOR:

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to participate in this research study.

---

Signature of Investigator

---

Date

**APPENDIX 8.2**

**NUTRITION SOURCE DOCUMENT:**

**ESTIMATED ENERGY REQUIREMENTS**

## Nutrition Source Document: Estimated Energy Requirements

**Date:** \_\_\_\_/\_\_\_\_/\_\_\_\_ **Completed by:** \_\_\_\_\_  
           day    month    year

**Participant initials:** \_\_\_\_\_ **Participant ID number:** \_\_\_\_\_ (4 digits)

- 1.) Weight (from Visit 1 source document) \_\_\_\_\_ kg
- 2.) Height (from Visit 1 source document) \_\_\_\_\_ cm
- 3.) Age (from Visit 1 source document) \_\_\_\_\_ years
- 4.) What are the participant's daily Estimated Energy Requirements? \_\_\_\_\_ kcal

EER (kcal) = ( number based on age and gender x body wt in kg ) + 300kcal

= ( \_\_\_\_\_ x \_\_\_\_\_ kg ) + 300kcal

= \_\_\_\_\_ (Rounded to nearest 100kcal)

- 5.) Number of daily test food servings to be consumed during randomization: \_\_\_\_\_

**Table 1 - Calculation for daily EER**

	Column 1	Column 2
<b>Age</b>	<b>Males</b>	<b>Females</b>
0 – 5	103.4	102.7
6 – 9	78.5	72.5
10 – 14	61.3	53.3
15 – 19	50.8	36.7
20 – 24	45.7	35.7
25 – 29	39.6	33.8
30 – 34	38.2	31.6
35 – 39	34.3	29.4
40 – 44	33.0	27.3
45 – 49	32.3	27.9
50 – 54	31.7	26.7
55 – 59	29.9	26.1
60 – 64	30.3	27.3
65 – 69	28.3	26.3
70 +	26.2	25.8

Calculation for Energy Expenditure Requirements (EER) based on a diet of 55% of total energy derived from carbohydrates (CHO) with 40% of available CHO provided from the test foods.  
 Total kcal/day x 0.55 = kcal from CHO  
 kcal of CHO divided by 4 kcal/g = total g CHO  
 total g CHO x 0.40 = grams CHO  
 grams CHO divided by 15 grams of CHO per test food serving = # test food servings  
 # test food servings are rounded to the nearest 0.5

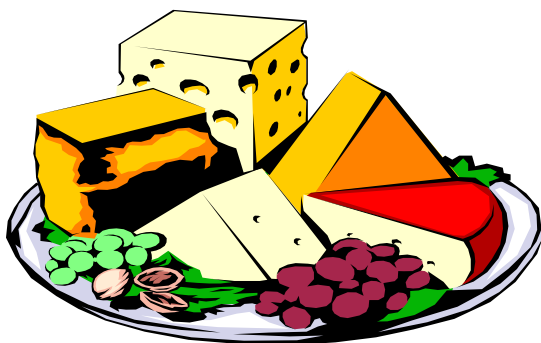
**Table 2 – Daily Test Food Requirements for Randomization**

<b>EER</b>	<b># Of Daily Test Food Servings*</b>	<b>EER</b>	<b># Of Daily Test Food Servings*</b>
1500	6	3100	12
1600	6	3200	12
1700	6.5	3300	12.5
1800	7	3400	13
1900	7	3500	13.5
2000	7.5	3600	14
2100	8	3700	14
2200	8.5	3800	14.5
2300	9	3900	15
2400	9	4000	15
2500	9.5	4100	15.5
2600	10	4200	16
2700	10.5	4300	16.5
2800	10.5	4400	17
2900	11	4500	17
3000	11.5		

**APPENDIX 8.3**  
**FACTS ON FAT INFORMATION SHEET**

# Facts on Fat

It is important to include fat in our daily diet but a diet that is too high in fat, especially bad fats, can lead to health problems. There is a lot to learn about fat and its role in health.



## WHAT IS FAT?

Fat is one of the three main components of food; the others are protein and carbohydrate. Fat has 9 Calories per gram compared to protein and carbohydrate at 4 Calories per gram. Individuals who eat a high fat diet regularly may be eating too many Calories and gain weight.

## UNSATURATED FAT

If you are going to ingest fat, then these are the types of fats that you should have because they are “*good fats*”. Unsaturated fats are usually listed on product labels as monounsaturated or polyunsaturated fats. Any type of fat that is liquid at room temperature is an unsaturated fat. Some unsaturated fats must be supplied by the diet because our body cannot make them. These important *good* fats are the omega-3 fats. They can be found in liquid oils, nuts and seeds, soybeans, flaxseed, fish and omega-3 products such as eggs. The omega-3 fats may help reduce your risk for developing heart disease.

## SATURATED FAT

These are the *BAD* fats. Saturated fats are found in meats, whole milk dairy products, coconut and palm oil, chocolate, processed foods, and fast foods. Saturated fat can increase risk for heart disease, so it is important that you keep your saturated fats to less than 10 % of your daily diet. By reducing the total amount of fat in your diet, you will usually decrease the saturated fats as well. The important thing to remember is that *saturated fats are the bad fats*.



## HYDROGENATION

Beware of hydrogenated fats because they are *bad fats*. Hydrogenation is a process that changes the good polyunsaturated fat to a bad saturated fat or “trans fat”. Trans fats are found in margarines, bakery products, snack foods, and fast foods. Foods that list hydrogenation or partially hydrogenated oils in the first 3 ingredients usually contain trans fats and saturated (bad) fats. *Trans fats are linked to heart disease and should be avoided*.

# Low Fat Diet

The recommended daily fat intake should be less than 30% of your total caloric intake. Here are some suggestions to help reduce the total amount of fat in your daily diet.

<b>Try to avoid the bad fats</b>	<b>Food Sources</b>
Saturated	whole milk, butter, poultry skin, fatty cuts of meat, coconut oil and palm oil, creamy salad dressings
“Trans fats”	shortening, hydrogenated margarine, crackers, cookies, cakes, pies, chips and other snack foods, processed foods
<b>Have more of the good fats</b>	<b>Food Sources</b>
Polyunsaturated and monounsaturated	Any liquid vegetable oil, fish, flax seed and flax oil, nuts and seeds, oil salad dressings

**Choose foods from all 4 food groups in Canada’s Food Guide to Healthy Eating.**

<b>Food Group</b>	<b># of Servings</b>	<b>Food Ideas (one serving)</b>
Milk Products	2-4 (for adults)	1 cup low fat milk or non-fat yogurt
Vegetables and Fruits	5-10	½ cup of vegetables, 1 fruit
Grain Products	5-12	1 slice of bread, ½ cup of pasta
Meat and Alternatives	2-3	1/3 cup tofu, ½ cup beans, 3 ½ oz of meat, fish or poultry

## Ways to reduce the fat in your diet:

- ☐ Drink skim or 1% milk
- ☐ Use less or try reduced-fat sauces, gravies, mayonnaise, cheeses and sour cream
- ☐ Use non-hydrogenated margarine rather than butter or regular margarine
- ☐ Cut extra fat off meat and remove skin and visible fat from poultry
- ☐ Broil, bake, boil or barbeque rather than frying foods
- ☐ Try salsa, mustard, chutneys, or spices to flavour foods rather than butter or margarine
- ☐ Have lettuce salads with oil or low-fat dressing instead of potato and macaroni salads
- ☐ Limit snack foods, baked goods, and deep-fried foods

## HEALTHY FAST FOOD CHOICES

**Wendys:** small chili, milk, salad with low fat dressing, (omit the fries)

**Tim Hortons:** juice, soup (water based), low fat muffin, coffee with milk

**McDonalds:** choose from the healthy choice menu, juice, milk (omit the fries)

**Subway:** choose from the 7 grams of fat or less menu, water, juice, milk,



**APPENDIX 8.4**  
**MEDFICTS DIETARY ASSESSMENT QUESTIONNAIRE**

### Sample Dietary Assessment Questionnaire

#### MEDFICTS\*

In each food category for both Group 1 and Group 2 foods check one box from the "Weekly Consumption" column (number of servings eaten per week) and then check one box from the "Serving Size" column. If you check Rarely/Never, do not check a serving size box. See next page for score.

Food Category	Weekly Consumption			Serving Size			Score	
	Rarely/ never	3 or less	4 or more	Small <5 oz/d 1 pt	Average 5 oz/d 2 pts	Large >5 oz/d 3 pts		
<b>Meats</b>								
<ul style="list-style-type: none"> <li>Recommended amount per day: ≤5 oz (equal in size to 2 decks of playing cards).</li> <li>Base your estimate on the food you consume most often.</li> <li>Beef and lamb selections are trimmed to 1/8" fat.</li> </ul> <p><b>Group 1.</b> 10g or more total fat in 3 oz cooked portion  <b>Beef</b> – Ground beef, Ribs, Steak (T-bone, Flank, Porterhouse, Tenderloin), Chuck blade roast, Brisket, Meatloaf (w/ground beef), Corned beef  <b>Processed meats</b> – 1/4 lb burger or lg. sandwich, Bacon, Lunch meat, Sausage/knockwurst, Hot dogs, Ham (bone-end), Ground turkey  <b>Other meats, Poultry, Seafood</b> – Pork chops (center loin), Pork roast (Blade, Boston, Sirloin), Pork spareribs, Ground pork, Lamb chops, Lamb (ribs), Organ meats<sup>1</sup>, Chicken w/skin, Eel, Mackerel, Pompano</p> <p><b>Group 2.</b> Less than 10g total fat in 3 oz cooked portion  <b>Lean beef</b> – Round steak (Eye of round, Top round), Sirloin<sup>1</sup>, Tip &amp; bottom round<sup>1</sup>, Chuck arm pot roast<sup>1</sup>, Top Loin<sup>1</sup>  <b>Low-fat processed meats</b> – Low-fat lunch meat, Canadian bacon, "Lean" fast food sandwich, Boneless ham  <b>Other meats, Poultry, Seafood</b> – Chicken, Turkey (w/o skin)<sup>1</sup>, most Seafood<sup>1</sup>, Lamb leg shank, Pork tenderloin, Sirloin top loin, Veal cutlets, Sirloin, Shoulder, Ground veal, Venison, Veal chops and ribs<sup>1</sup>, Lamb (whole leg, loin, fore-shank, sirloin)<sup>1</sup></p>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	x	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
		3 pts	7pts		1 pt	2 pts	3 pts	
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	x	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
							6 pts	
<b>Eggs</b> – Weekly consumption is the number of times you eat eggs each week								
Check the number of eggs eaten each time								
<b>Group 1.</b> Whole eggs, Yolks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	x	≤1 1 pt	2 2 pts	≥3 3 pts	_____
		3 pts	7pts					
<b>Group 2.</b> Egg whites, Egg substitutes (1/2 cup)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>Dairy</b>								
<b>Milk</b> – Average serving 1 cup				x	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>Group 1.</b> Whole milk, 2% milk, 2% buttermilk, Yogurt (whole milk)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		1 pt	2 pts	3 pts	
		3 pts	7pts					
<b>Group 2.</b> Fat-free milk, 1% milk, Fat-free buttermilk, Yogurt (fat-free, 1% low fat)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>Cheese</b> – Average serving 1 oz				x	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>Group 1.</b> Cream cheese, Cheddar, Monterey Jack, Colby, Swiss, American processed, Blue cheese, Regular cottage cheese (1/2 cup), and Ricotta (1/4 cup)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		1 pt	2 pts	3 pts	
		3 pts	7pts					
<b>Group 2.</b> Low-fat & fat-free cheeses, Fat-free milk mozzarella, String cheese, Low-fat, Fat-free milk & Fat-free cottage cheese (1/2 cup) and Ricotta (1/4 cup)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>Frozen Desserts</b> – Average serving 1/2 cup				x	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>Group 1.</b> Ice cream, Milk shakes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		1 pt	2 pts	3 pts	
		3 pts	7pts					
<b>Group 2.</b> Low-fat ice cream, Frozen yogurt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____

FIG MEDFICTS assessment tool.

\* MEDFICTS was originally developed for and printed in ATP II<sup>12</sup>

**Sample Dietary Assessment Questionnaire (Continued)**  
**MEDFICTS\***

Food Category	Weekly Consumption			x	Serving Size			Score
	Rarely/ never	3 or less	4 or more		Small <5 oz/d 1 pt	Average 5 oz/d 2 pts	Large >5 oz/d 3 pts	
<b>Frying Foods</b> – Average servings: see below. This section refers to method of preparation for vegetables and meat.								
<b>Group 1.</b> French fries, Fried vegetables (1/2 cup), Fried chicken, fish, meat (3 oz)	<input type="checkbox"/>	<input type="checkbox"/> 3 pts	<input type="checkbox"/> 7pts		<input type="checkbox"/> 1 pt	<input type="checkbox"/> 2 pts	<input type="checkbox"/> 3 pts	_____
<b>Group 2.</b> Vegetables, not deep fried (1/2 cup), Meat, poultry, or fish—prepared by baking, broiling, grilling, poaching, roasting, stewing: (3 oz)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>In Baked Goods</b> – 1 Average serving								
<b>Group 1.</b> Doughnuts, Biscuits, Butter rolls, Muffins, Croissants, Sweet rolls, Danish, Cakes, Pies, Coffee cakes, Cookies	<input type="checkbox"/>	<input type="checkbox"/> 3 pts	<input type="checkbox"/> 7pts		<input type="checkbox"/> 1 pt	<input type="checkbox"/> 2 pts	<input type="checkbox"/> 3 pts	_____
<b>Group 2.</b> Fruit bars, Low-fat cookies/cakes/pastries, Angel food cake, Homemade baked goods with vegetable oils, breads, bagels	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>Convenience Foods</b>								
<b>Group 1.</b> Canned, Packaged, or Frozen dinners: e.g., Pizza (1 slice), Macaroni & cheese (1 cup), Pot pie (1), Cream soups (1 cup), Potato, rice & pasta dishes with cream/cheese sauces (1/2 cup)	<input type="checkbox"/>	<input type="checkbox"/> 3 pts	<input type="checkbox"/> 7pts		<input type="checkbox"/> 1 pt	<input type="checkbox"/> 2 pts	<input type="checkbox"/> 3 pts	_____
<b>Group 2.</b> Diet/Reduced calorie or reduced fat dinners (1), Potato, rice & pasta dishes without cream/cheese sauces (1/2 cup)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>Table Fats</b> – Average serving: 1 Tbsp								
<b>Group 1.</b> Butter, Stick margarine, Regular salad dressing, Mayonnaise, Sour cream (2 Tbsp)	<input type="checkbox"/>	<input type="checkbox"/> 3 pts	<input type="checkbox"/> 7pts		<input type="checkbox"/> 1 pt	<input type="checkbox"/> 2 pts	<input type="checkbox"/> 3 pts	_____
<b>Group 2.</b> Diet and tub margarine, Low-fat & fat-free salad dressing, Low-fat & fat-free mayonnaise	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____
<b>Snacks</b>								
<b>Group 1.</b> Chips (potato, corn, taco), Cheese puffs, Snack mix, Nuts (1 oz), Regular crackers (1/2 oz), Candy (milk chocolate, caramel, coconut) (about 1 1/2 oz), Regular popcorn (3 cups)	<input type="checkbox"/>	<input type="checkbox"/> 3 pts	<input type="checkbox"/> 7pts		<input type="checkbox"/> 1 pt	<input type="checkbox"/> 2 pts	<input type="checkbox"/> 3 pts	_____
<b>Group 2.</b> Pretzels, Fat-free chips (1 oz), Low-fat crackers (1/2 oz), Fruit, Fruit rolls, Licorice, Hard candy (1 med piece), Bread sticks (1–2 pcs), Air-popped or low-fat popcorn (3 cups)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	_____

† Organ meats, shrimp, abalone, and squid are low in fat but high in cholesterol.

‡ Only lean cuts with all visible fat trimmed. If not trimmed of all visible fat, score as if in Group 1.

¥ Score 6 pts if this box is checked.

§ All parts not listed in group 1 have &lt;10g total fat.

Total from page 1 \_\_\_\_\_

Total from page 2 \_\_\_\_\_

**Final Score** \_\_\_\_\_

**To Score:** For each food category, multiply points in weekly consumption box by points in serving size box and record total in score column. If Group 2 foods checked, no points are scored (except for Group 2 meats, large serving = 6 pts).

**Example:**

<input type="checkbox"/>	<input type="checkbox"/> 3 pts	<input checked="" type="checkbox"/> 7 pts	x	<input type="checkbox"/> 1 pt	<input type="checkbox"/> 2 pts	<input checked="" type="checkbox"/> 3 pts	_____
--------------------------	-----------------------------------	--	---	----------------------------------	-----------------------------------	--	-------

Add score on page 1 and page 2 to get final score.

**Key:**

≥70 Need to make some dietary changes

40–70 Heart-Healthy Diet

&lt;40 TLC Diet

FIG. MEDFICTS assessment tool.

\* MEDFICTS was originally developed for and printed in ATP II<sup>1,2</sup>

**APPENDIX 8.5**  
**3-DAY FOOD DIARIES**



### 3 DAY FOOD DIARY: PARTICIPANT INSTRUCTIONS

Participant initials: \_\_\_\_\_ Participant ID#: \_\_\_\_\_

- Please maintain your usual eating habits.
- Complete the food record the week before your next appointment.
- Use a new diet record form for each day. It may be helpful for dietary input if the participant attaches labels from the products eaten to the 3-day food diary. The labels can be attached to page 2 of each day.
- Write down **everything** you eat and drink for 2 weekdays and 1 weekend day (whenever possible). Try and record the food and beverage items immediately after you have consumed them. Remember to include the time at which the food was eaten.
- Use measuring cups, tablespoons, teaspoons to measure or a scale to weigh out the quantities of food. **The more accurate the better!!**
- Describe the food in as much detail as possible. Specify item, brand, and amount.
  - ½ cup Delmonte fruit cocktail canned in light syrup
  - 2 tsp Becel regular margarine
  - 45 g (1 large slice) of Dempsters 7 grain bread
- Write down the percent fat in milk products (i.e. %MF, or %BF).
  - 8 oz 1% milk
  - 175 g 1% Astro fruit bottom peach yogurt
- Include items added to food and drinks.
  - i.e. sugar, cream, ketchup, dressings, sauces, toppings, spreads, etc.
- Include on your food record the fat used when preparing foods.
  - i.e. Butter, canola oil, etc.
- Write down the name of the restaurant if you are eating out.
- Describe the method of cooking.
  - Steaming, broiling, pan-fried, deep-fried.

#### Examples

##### Correct

1 medium bagel  
1 teaspoon regular Becel Margarine  
2 ounces of lean ham  
1-ounce medium cheddar cheese (33% MF)  
1 tsp mustard  
1 lettuce leaf (Romaine lettuce)

##### Incorrect

1 ham and cheese sandwich

##### Correct

1 ½ cup cooked white rice (Uncle Ben's)  
2 cups stir fried vegetables

- ½ zucchini
- ¼ red pepper
- 3 mushrooms
- ½ cup cooked broccoli

2-ounce boneless, skinless chicken breast

- 1 tsp cornstarch
- 1 Tbsp soy sauce
- 1 Tbsp teriyaki sauce
- 1 Tbsp canola oil

##### Incorrect

rice with chicken and stir-fried vegetables

3-DAY FOOD DIARY Dispensed Visit # \_\_\_\_ Returned Visit # \_\_\_\_

**Participant initials:** \_\_\_\_\_ **Participant ID#:** \_\_\_\_\_

## Day 1

**Date:** \_\_\_\_/\_\_\_\_/\_\_\_\_  
day month year

Is this a usual day? \_\_\_\_\_no \_\_\_\_\_yes

[illegible]

## 3-DAY FOOD DIARY Dispensed Visit # \_\_\_\_ Returned Visit # \_\_\_\_

**Participant initials:** \_\_\_\_\_ **Participant ID#:** \_\_\_\_\_

**Day 2**      **Date:**        /        /         
                                 day      month      year      Is this a usual day?          no          yes

[illegible]



## Day 3

Is this a usual day? \_\_\_\_\_no \_\_\_\_\_yes

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**APPENDIX 8.6**  
**BAECKE PHYSICAL ACTIVITY QUESTIONNAIRE**

## APPENDIX

## Questionnaire, codes, and method of calculation of scores on habitual physical activity

1) What is your main occupation?	1-3-5	
2) At work I sit never/seldom/sometimes/often/always	1-2-3-4-5	
3) At work I stand never/seldom/sometimes/often/always	1-2-3-4-5	
4) At work I walk never/seldom/sometimes/often/always	1-2-3-4-5	
5) At work I lift heavy loads never/seldom/sometimes/often/very often	1-2-3-4-5	
6) After working I am tired very often/often/sometimes/seldom/never	5-4-3-2-1	
7) At work I sweat very often/often/sometimes/seldom/never	5-4-3-2-1	
8) In comparison with others of my own age I think my work is physically much heavier/heavier/as heavy/lighter/much lighter	5-4-3-2-1	
9) Do you play sport? yes/no		
If yes:		
— which sport do you play most frequently?		Intensity 0.76 — 1.26 — 1.76
— how many hours a week?		Time 0.5 — 1.5 — 2.5 — 3.5 — 4.5
— how many months a year?		Proportion 0.04 — 0.17 — 0.42 — 0.67 — 0.92
If you play a second sport:		
— which sport is it?		Intensity 0.76 — 1.26 — 1.76
— how many hours a week?		Time 0.5 — 1.5 — 2.5 — 3.5 — 4.5
— how many months a year?		Proportion 0.04 — 0.17 — 0.42 — 0.67 — 0.92
10) In comparison with others of my own age I think my physical activity during leisure time is much more/more/the same/less/much less	5-4-3-2-1	
11) During leisure time I sweat very often/often/sometimes/seldom/never	5-4-3-2-1	
12) During leisure time I play sport never/seldom/sometimes/often/very often	1-2-3-4-5	
13) During leisure time I watch television never/seldom/sometimes/often/very often	1-2-3-4-5	
14) During leisure time I walk never/seldom/sometimes/often/very often	1-2-3-4-5	
15) During leisure time I cycle never/seldom/sometimes/often/very often	1-2-3-4-5	
16) How many minutes do you walk and/or cycle per day to and from work, school and shopping? <5/5-15/15-30/30-45/>45	1-2-3-4-5	
Calculation of the simple sport-score ( $I_9$ ): (a score of zero is given to people who do not play a sport)		
$I_9 = \sum_{i=1}^{16} (\text{intensity} \times \text{time} \times \text{proportion})$		
$= 0/0.01 - <4/4 - <8/8 - <12/12 \geq 12$		
Calculation of scores of the indices of physical activity:		
Work index = $[(I_1 + (6 - I_2) + I_3 + I_4 + I_5 + I_6 + I_7 + I_8)/8]$		
Sport index = $[(I_9 + I_{10} + I_{11} + I_{12})/4]$		
Leisure-time index = $[(6 - I_{13}) + I_{14} + I_{15} + I_{16})/4]$		

**APPENDIX 8.7**

**LOW GLYCEMIC INDEX AND HIGH GLYCEMIC INDEX WEEKLY**

**TEST FOOD DIARIES**

## Weekly Test Food Diary

### Low GI diet

Initials: \_\_\_\_\_ ID#: \_\_\_\_\_

Dispensed Visit #: \_\_\_\_\_ Returned Visit #: \_\_\_\_\_

Start Date: \_\_\_\_\_ Finish Date: \_\_\_\_\_

Please have \_\_\_\_\_ servings of the test foods every day.

= \_\_\_\_\_ for \_\_\_\_\_ days

**Compliance %** = # of test foods consumed \_\_\_\_\_

divided by # of test foods required \_\_\_\_\_ X 100 = \_\_\_\_\_

- Record the intake of the test foods as soon as possible after consumption.
- Ensure that at least **two** servings are with the first meal of the day.
- Measure all of the foods as precisely as possible (use measuring cups and measuring spoons provided)
- Indicate the number of servings of each test food consumed.  
(one "x" will equal one serving for each food)

***Each measurement is one serving unless otherwise specified.***



ID#: \_\_\_\_\_ Initials: \_\_\_\_\_

Week #: \_\_\_\_\_ Week starting: \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_  
 Day Month Year

Low Glycemic Index Diet								Serving Total
Bran Buds with Psyllium (1/3 cup)								
Raisin Bran (dry) (1/2 cup)								
Oatmeal (non-instant) (1/2 cup cooked, 1/3 cup dry)								
Red River Cereal (1/2 cup cooked, 1/6 cup dry)								
Pumpernickel bread Dimpflmeier 1 slice = 1 1/2 servings								
Pumpernickel bread Holtzheuser 1 slice = 1 1/2 servings								
Linseed bread Dimpflmeier 1 slice = 1 1/2 servings								
100% Rye bread Vollkornbrot 1 slice = 1 1/2 servings								
Barley (1/4 cup dry or 1/2 cup cooked = 2 servings)								
Pasta (al dente) (3/4 cup cooked or 1/3 cup dry = 2 servings)								
Parboiled Rice (1/3 cup cooked)								
Bulgar (1/2 cup cooked)								
Beans: canned, kidney, navy, white, cooked (1/2 cup)								
Beans, canned Brown, baked, plain (1/2 cup)								
Beans, Chickpeas, canned (3/4 cup = 2 servings)								
Lentils: red, brown, green, (1/2 cup cooked)								
PC Instant Black Bean Soup (dry) (1/2 portion or 34g)								
PC Instant Vegetable Barley soup (dry) (1/2 portion or 23g)								
PC Instant Lentil Soup (dry) (1/2 portion or 33g)								
Habitant Split Pea Soup, canned (2/3 cup)								
<b>SERVING TOTAL</b>								

**Weekly Test Food Diary**  
**Control diet**

Initials: \_\_\_\_\_ ID#: \_\_\_\_\_

Dispensed Visit #: \_\_\_\_\_ Returned Visit #: \_\_\_\_\_

Start Date: \_\_\_\_\_ Finish Date: \_\_\_\_\_

Please have \_\_\_\_\_ servings of the test foods every day.

= \_\_\_\_\_ for \_\_\_\_\_ days

**Compliance %** = # of test foods consumed \_\_\_\_\_

divided by # of test foods required \_\_\_\_\_ X 100 = \_\_\_\_\_

- Record the intake of the test foods as soon as possible after consumption.
- Ensure that at least **two** servings are with the first meal of the day.
- Measure all of the foods as precisely as possible (use measuring cups and measuring spoons provided)
- Indicate the number of servings of food consumed. (put an “x” for each serving of food in the appropriate box)

***Each measurement is one serving unless otherwise specified.***



Initials: \_\_\_\_\_

ID#: \_\_\_\_\_

Week #: \_\_\_\_\_ Week of: \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_

Day

Month

Year

Control Diet (High Glycemic Index Diet)								Serving Total
President's Choice Cornflakes (2/3 cup)								
Crispex Cereal (2/3 cup)								
Corn Bran Cereal (2/3 cup)								
Cheerios cereal General Mills (2/3 cup)								
Shredded Wheat (1 large or 1/2 cup)								
Cream Of Wheat (1/2 cup cooked)								
Melba Toast (4 each)								
White Bread (1 slice)								
Whole Wheat Bread 60% (1 slice)								
Whole Wheat Bread 100% (1 slice)								
Light Rye Bread (1 slice)								
Soda Crackers (7 each)								
Graham Crackers (3 each)								
Stoned Wheat Thins (3 each)								
White Polished Rice Cooked (1/3 cup)								
Brown Rice Cooked (1/3 cup)								
Instant Potatoes Cooked (1/2 cup)								
Home-made mashed potatoes, cooked (1/2 cup)								
Idaho Baked Potato (1/2 medium or 1/2 cup)								
Instant Potato and Leek Soup (1/2 package or 17 g)								
<b>SERVING TOTAL</b>								